

COLUMBIA LIBRARIES OFFSITE
HEALTH SCIENCES RESTRICTED



HR01615475

SERIAL

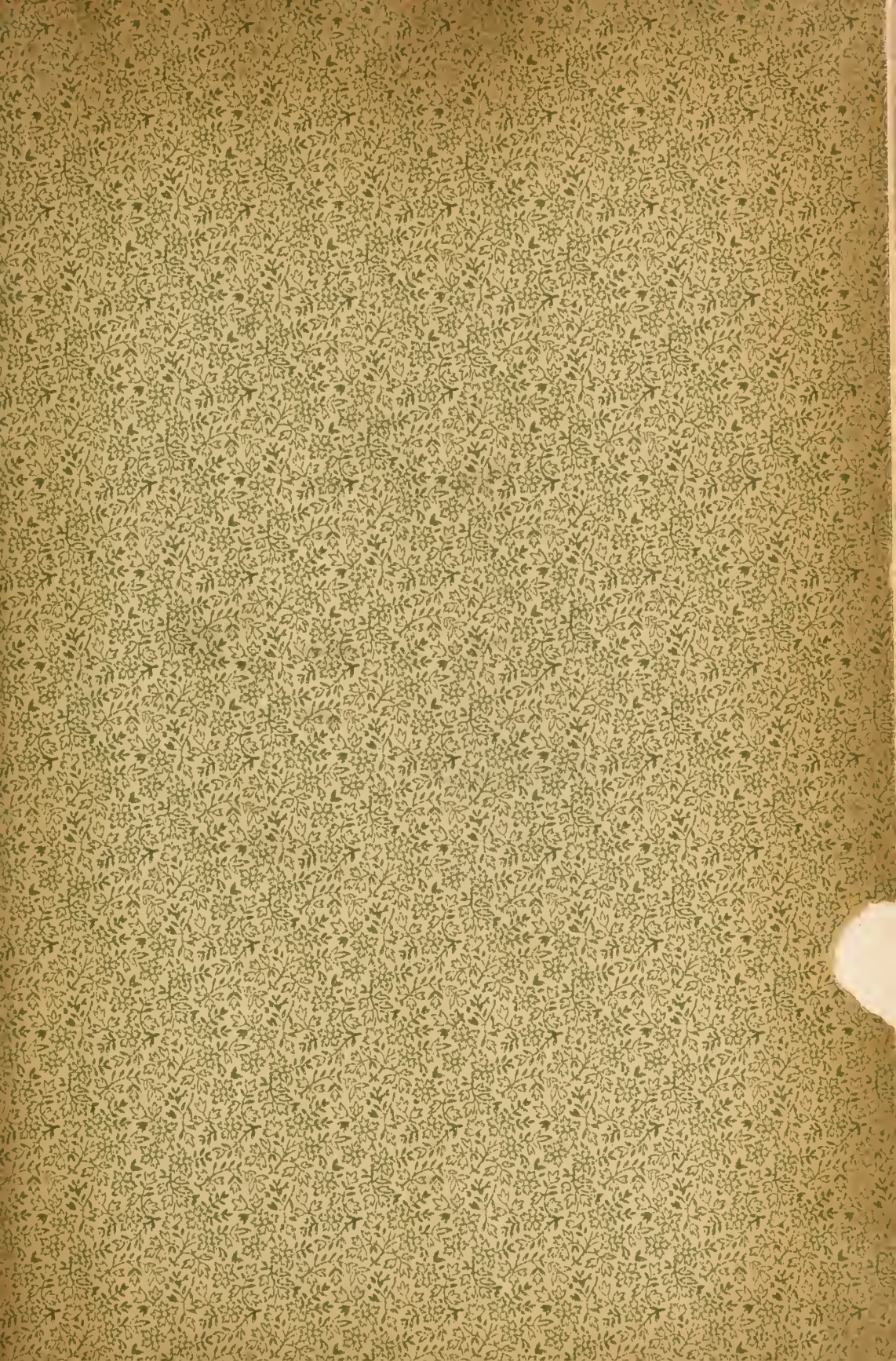
J

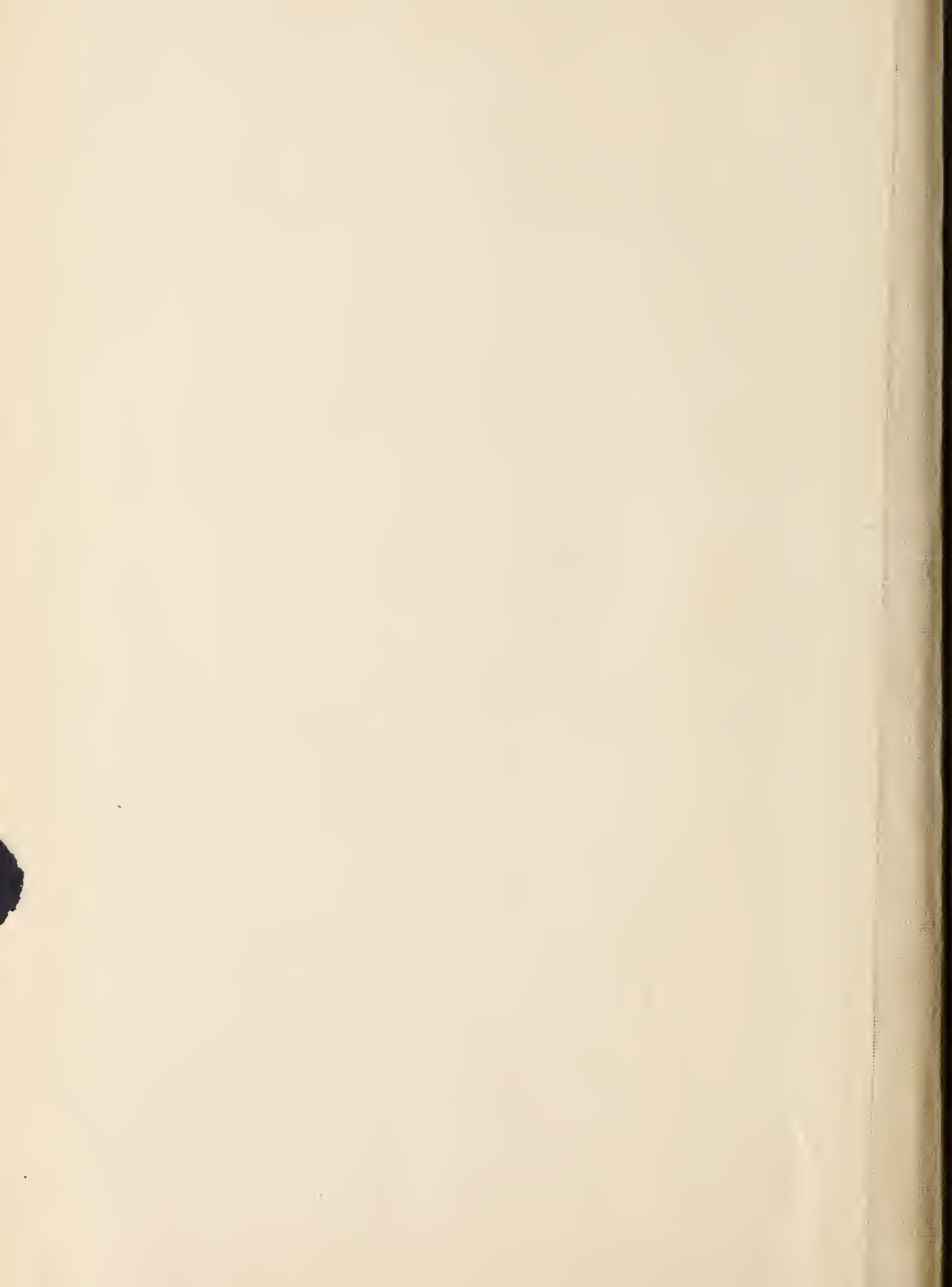
V.16
1915

cop.1



COLUMBIA UNIVERSITY
EDWARD G. JANEWAY
MEMORIAL LIBRARY







Digitized by the Internet Archive
in 2014

<https://archive.org/details/journalofinfecti1619memo>

THE
JOURNAL OF INFECTIOUS DISEASES

The
Journal of Infectious Diseases

Published by the Memorial Institute for Infectious Diseases

EDITED BY
LUDVIG HEKTOEN AND EDWIN O. JORDAN

IN CONJUNCTION WITH
FRANK BILLINGS F. G. NOVY
W. T. SEDGWICK H. GIDEON WELLS

Volume 16
1915

Chicago, 1915

Composed and Printed by
American Medical Association Press
Chicago, Illinois, U. S. A.

TABLE OF CONTENTS

NO. 1, JANUARY, 1915

Typhoid Fever in Rockford, Illinois	PAGE
<i>Paul Hansen and Horatio N. Parker</i> - - - - -	1
The Effect of Quinin on Rabies	
<i>Charles Krumwiede, Jr., and Alice G. Mann</i> - - - - -	24
On the Relative Virulence of Sensitized and Non-Sensitized Typhoid Bacilli	
<i>Russell L. Cecil</i> - - - - -	26
Classification of the Bacillus Welchii Group of Bacteria	
<i>J. P. Simonds</i> - - - - -	31
The Effect of Symbiosis upon Spore Formation by Bacillus Welchii, with Special Reference to the Presence of these Spores in Stools	
<i>J. P. Simonds</i> - - - - -	35
The Action of Sodium Sulphocyanate in Tuberculosis. Studies on the Biochemistry and Chemotherapy of Tuberculosis, XII	
<i>Harry J. Corper</i> - - - - -	38
Sodium Tellurite as a Rapid Test for the Viability of Tubercle Bacilli. Studies on the Biochemistry and Chemotherapy of Tuberculosis, XIII	
<i>Harry J. Corper</i> - - - - -	47
Inoculation Experiment with Pure Culture of Spirochaeta Hyos. Studies on Hog-Cholera	
<i>Walter E. King and Raymond H. Drake</i> - - - - -	54
Further Observations on the Effect of Quinin in Rabies	
<i>V. H. Moon</i> - - - - -	58
On the Specific Precipitin in the Blood of Persons Injected with Anti- diphtheric Horse Serum	
<i>Clifford W. Wells</i> - - - - -	63
The Fecal Flora of Typhoid Fever and Its Reaction to Various Diets	
<i>John C. Torrey</i> - - - - -	72

No. 2, MARCH, 1915

The Mechanism of Phagocytosis	
<i>G. L. Kite and W. B. Wherry</i> - - - - -	109
The Technic of the Wassermann Reaction with Reference to Thomas and Ivy's Method of Complement Dosage and to the Management of Natural Antisheep Amboceptor	
<i>Reuben Ottenberg and Blanche Frazier</i> - - - - -	119

	PAGE
A Comparison of the Immunizing Effects of the Subcutaneous and Intra-peritoneal Administrations of Tumor Cells Against the Growth of Carcinoma in Mice <i>M. G. Seelig and Moyer S. Fleisher</i> - - - - -	122
Experimental Study in the Distribution and Habitat of the Tetanus Bacillus <i>Willis Noble</i> - - - - -	132
Syphilitic Leptomeningitis (<i>with Plates 1-11</i>) <i>E. R. LeCount and Kaethe Dewey</i> - - - - -	142
Studies on the Cultivation of the Virus of Vaccinia III, with a Note on the Glycerin Resistance of Various Organisms <i>Edna Steinhardt and Marie Grund</i> - - - - -	205
A Study of the So-Called Implantation of the Bacillus Bulgaricus <i>Alfred H. Rahe</i> - - - - -	210
The Variability of Two Strains of Streptococcus Lacticus <i>P. G. Heinemann</i> - - - - -	221
The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli (<i>with Plates 12-16</i>) <i>Edward C. Rosenow</i> - - - - -	240
On the Spirochetel Infection of Ulcers in China <i>H. E. Eggers</i> - - - - -	269
Relation of the Number of Streptococcus Lacticus to the Amount of Acid Formed in Milk and Cream <i>P. G. Heinemann</i> - - - - -	285
Bacteriemia Due to Bacillus Diphtheriae <i>H. Windsor Wade</i> - - - - -	292
Complement Fixation in the Diagnosis of Gonococcal Infections <i>Ernest E. Irons and H. K. Nicoll</i> - - - - -	303
A Method of Transmitting Blood Parasites <i>John A. Kolmer</i> - - - - -	311
The Production, Through Immunization, of Specific Ferments Against Bacteria as Detected by the Abderhalden Test <i>George H. Smith</i> - - - - -	313
The Production and Detection of Specific Ferments for the Typhoid-Coli Group <i>George H. Smith</i> - - - - -	319
A Study of the Correlation of the Agglutination and the Fermentation Reactions Among the Streptococci <i>I. J. Kligler</i> - - - - -	327
The Diagnostic Value of Intracutaneous Injection of Diphtheria Toxin (Schick Reaction) <i>George H. Weaver and Loretta Maher</i> - - - - -	342

TABLE OF CONTENTS

vii

NO. 3, MAY, 1915

	PAGE
The Influence of an Oxidizing Substance (Sodium Iodoxybenzoate) on Immune Reactions	
<i>Aaron Arkin</i> - - - - -	349
Simultaneous Infection in a Child with Tubercle Bacilli of the Human and of the Bovine Type	
<i>Arent de Besche</i> - - - - -	361
The Etiology and Experimental Production of Erythema Nodosum (<i>with Plates 17-22</i>)	
<i>Edward C. Rosenow</i> - - - - -	367
Starch Agar a Useful Culture Medium	
<i>Edward B. Vedder</i> - - - - -	385
Complement Fixation in Whooping Cough	
<i>Walter Winholt</i> - - - - -	389
Various Sporotricha Differentiated by the Fermentation of Carbohydrates. Studies on American Sporotrichosis, I	
<i>K. F. Meyer and J. A. Aird</i> - - - - -	399
Individual and Group Variation in Guinea-Pigs in the American Method of Testing Tetanus Antitoxin	
<i>Loren B. Taber</i> - - - - -	410
Studies on the Gonococcus, III	
<i>Carl C. Warden</i> - - - - -	426
Natural Hemolysins in Human Serum	
<i>John A. Kolmer and Arthur J. Casselman</i> - - - - -	441
An Epidemic, Simulating Typhoid, Caused by a Paragaertner Organism	
<i>George H. Robinson</i> - - - - -	448
Complement Fixation in Acute Rhinitis	
<i>Katharine Howell</i> - - - - -	456
Conglutination in the Diagnosis of Dourine (Trypanosomiasis of the Horse)	
<i>Heinrich Wehrbein</i> - - - - -	461
The Ferment Activity of the Blood Serum in Infectious Diseases	
<i>Frederick Howard Falls</i> - - - - -	466
The Germicidal Effect of Lactic Acid in Milk	
<i>P. G. Heinemann</i> - - - - -	479
The Vacuum Method of Drawing Antihog Cholera Serum	
<i>Thos. P. Haslam, A. E. Hagan, and R. V. Christian</i> - - - - -	487
Further Observations on the Bacteriology of Rhinitis with Special Reference to an Anaerobic Organism (<i>Bacillus Rhinitis</i>)	
<i>Ruth Tunnichliff</i> - - - - -	493

The Journal of Infectious Diseases

PUBLISHED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. 16

January 1915

NO. 1

TYPHOID FEVER IN ROCKFORD, ILLINOIS *

PAUL HANSEN AND HORATIO N. PARKER

(From the University of Illinois, Urbana, Illinois)

Rockford is a manufacturing city in northern Illinois, on Rock River, and has a population of 51,500. There is no distinct housing problem, and there seems to be no reason why the general health of the city should not be good, particularly as the Rockford Health Department is of more than average efficiency. Yet, there have been three well-marked typhoid fever epidemics within the last twenty months.

The first of these was studied by Jordan and Irons.¹ They found that the Rockford outbreaks of enteritis (10,000 cases) in January, and of typhoid fever (199 cases) in February, 1912, were due to an unusual contamination of the public water-supply during a factory conflagration on January 16, 1912. This was an unexpected conclusion in view of the fact that the supply was derived from very deep rock wells, the water from which, upon analysis, had hitherto proved of excellent quality. These investigations, however, revealed grave dangers of contamination in handling the water under heavy draught, such as actually occurred on January 16. These dangers included:

* Received for publication October 20, 1914.

1. Jour. Infect. Dis., 1912, 11, p. 21.

(1) A well held in reserve for fire emergency at the top of which was a receiving basin subject to the inflow of street dirt and, possibly, leakage from a nearby sewer; (2) a leaky collecting storage reservoir with privies close to its walls; (3) a leaky gravity conduit between emergency wells and the reservoir; (4) leaky pump-pits in an open, gravelly formation and within fifty feet of the sewage-polluted Rock River, and (5) inadequately protected connections between the city mains and factory sprinkler systems supplied also from water pumped from the polluted Rock River. Which one, or combination, of these elements was responsible for the epidemic could not be definitely ascertained.

Jordan, at the close of his investigation, warned the public that secondary outbreaks were likely to occur inasmuch as carriers and other sources of infection had become established in the city.

After the big epidemic of 1912, typhoid fever gradually became less common in Rockford until May, 1913, when no cases were reported. However, in July, August, September, and October, 1913, the disease reappeared, and the Health Commissioner, Dr. W. E. Parks, with Jordan's warning in mind, appealed to the State Board of Health for expert advice on the situation. Dr. C. E. Crawford was detailed to the investigation and called to his assistance Paul Hansen, engineer of the State Water Survey, and H. N. Parker of the division of Dairy Bacteriology of the University of Illinois.

GENERAL OBSERVATIONS

The distribution of cases by months during 1912 and 1913 is shown in Diagram 1. The enormous preponderance of cases in February, 1912, was unquestionably due to the contamination of the public water-supply, and the persistencé of typhoid thereafter is explained by the fact that Rockford was seeded with typhoid at the time of the epidemic and, as a result, there were numerous local outbreaks. Yet, except for a marked rise owing to a milk epidemic in November, 1912, amongst the customers (thirty-six cases) of one milk dealer, there was an irregular decline in the number of cases to May, 1913, when the city was free from typhoid. The occurrence of eleven cases in July caused some uneasiness, and when these were followed by fifty-one cases in August and fifty-nine cases in September, there developed a feeling of general alarm. When this inquiry closed on November 1, the record showed one hundred and sixty-five cases after June 1, all but nine of which occurred after July 1.

Magnitude of the Outbreak.—In 1913, there were one hundred and seventy-three cases of typhoid and sixteen deaths from it, giving a morbidity rate of 335 and a mortality rate of 31 per 100,000, which may be considered excessive.

Relation to Temperature.—Sedgwick and Winslow have shown that prosodemic typhoid closely follows the rise and fall of mean monthly temperatures, and that there is a striking coincidence in the curves of mean monthly temperature and monthly death-rates, but

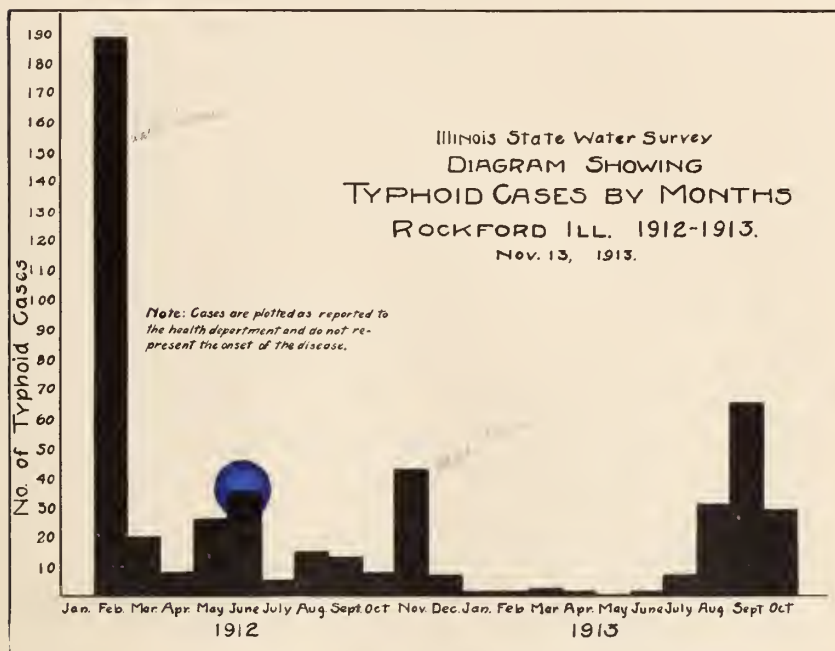


Diagram 1.

that the curves depart widely from each other in cities where epidemics occur, more particularly when the epidemics are due to polluted water-supplies (see Diagram 2).

In order to analyze the occurrence of typhoid fever in Rockford in the light of these deductions, Diagram 3 has been prepared showing the monthly variation in temperature and the number of cases that developed each month. From the diagram, it appears that the distribution of cases in 1912 was abnormal, whereas in 1913 it roughly

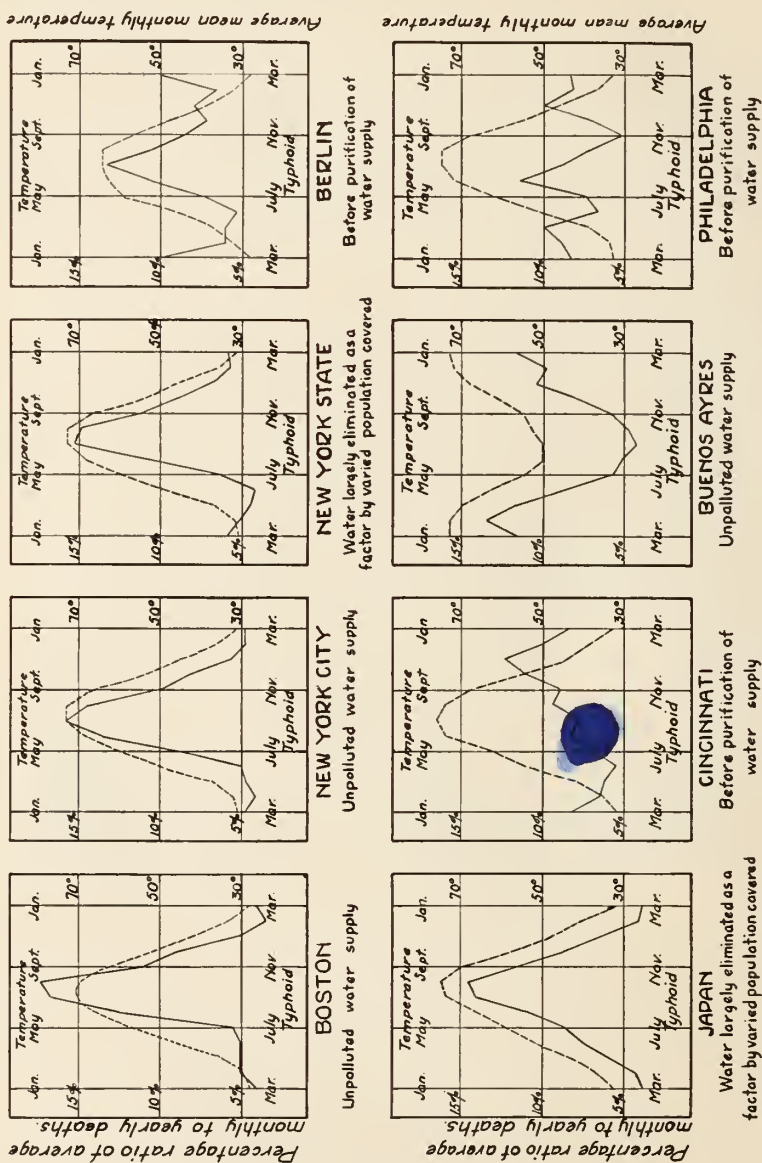


DIAGRAM ILLUSTRATING RELATION OF TYPHOID FEVER TO TEMPERATURE AFTER SEDGWICK AND WINSLOW.

approximated the normal. It should be remembered that an epidemic of typhoid may occur when the prosodemic typhoid is at a maximum. To determine whether or not there has been such a concurrence, the reported cases must be studied in detail.

It is to be emphasized that the appearance of so many cases in Rockford during summer and autumn would lead one to expect a number of cases of miscellaneous and vague origin that cannot be definitely explained. This was precisely the condition encountered.

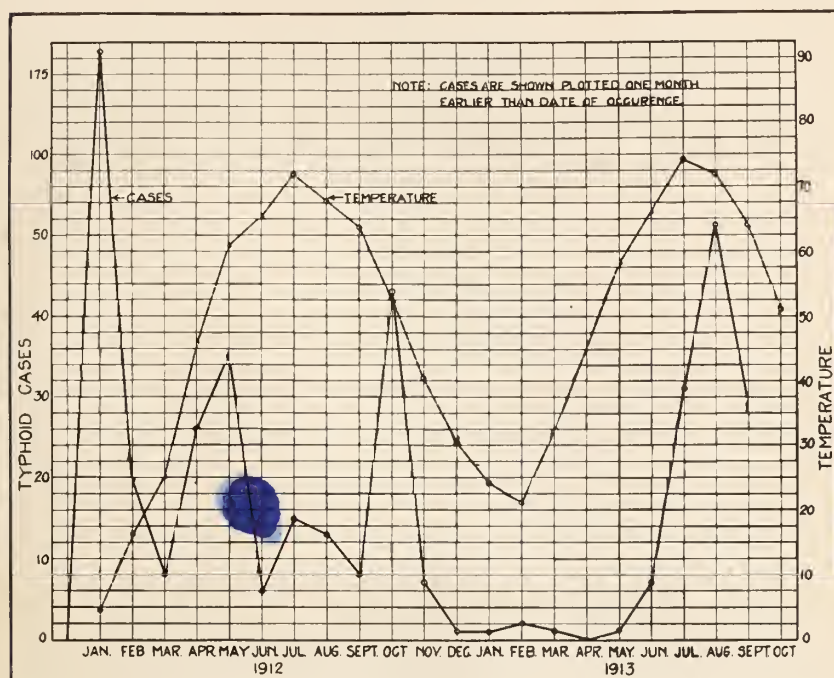


Diagram 3.—Showing monthly variation in temperature and the number of cases that developed each month.

Some well-marked groups of cases have been assigned a cause, but there remains a large number of cases for which the mode of infection is unknown. Because of these obscurities and complexities, this epidemic of 1913 was much more difficult to study than the epidemic of 1912, which was explosive in character and due to a single cause.

Daily Distribution of Typhoid.—Diagram 4 shows the occurrences of typhoid fever cases day by day during July, August, September,

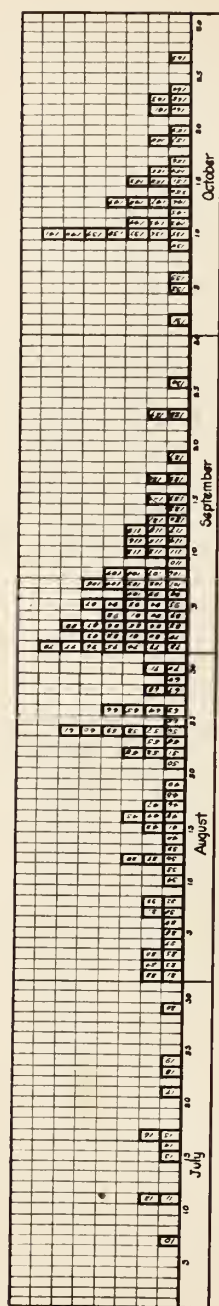


Diagram 4.—Cases day by day during July, August, September and October. Each rectangle represents a case and so placed according to the date at which the patient took to bed. The number within the rectangle designates the number of the case.

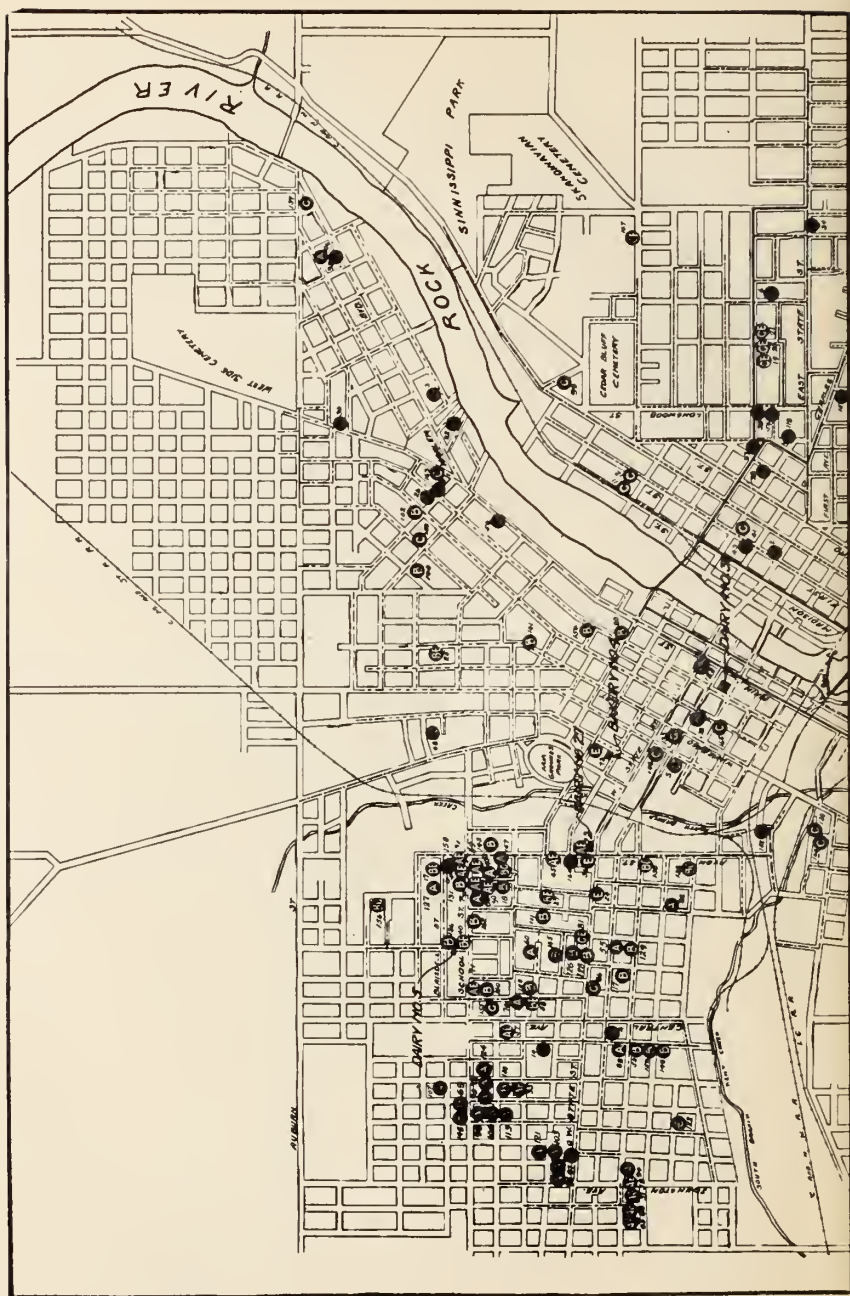
and October, 1913; each rectangle represents a case and is placed according to the date at which the patient took to bed. The numbers within the rectangles designate the number of the case and were used in studying the data. The diagram makes it evident that most of the cases occurred in August and the first two and one-half weeks in September, with a marked number of cases in the middle of October. It suggests that a cause, or that causes, of typhoid fever became active early in July, worked with increasing effect until the beginning of the last week in August, and then subsided rather rapidly in the next two weeks. Apparently, infection started anew in the last week in September and remained active for a few days. These conclusions are about all that one is warranted to draw from Diagram 4, but more illuminating evidence may be secured by grouping the cases. Before attempting this, certain other points should be considered.

Geographical Distribution of Cases.—Diagram 5 is a spot map of the city. Each dot shows a case of typhoid fever and is numbered to correspond with a rectangle in Diagram 4. On the map appear the dairies, public water mains, and the connections between these mains and the factory fire-fighting systems.

The cases are scattered throughout the built-up portion of the city, but there is a tendency for them to concentrate in the northwestern part, which is a medium to high-class residential district situated on high ground, and for them to become thicker in the eastern and southeastern sections of the city. From the diagram, it may be deduced that agents of infection have been at work throughout the city, and that something additional has been operative to make infection particularly active in the sections of the city noted.

Explanation of the cause of typhoid fever scattered over an entire city is usually sought in the public water-supply or in those miscellaneous factors that are active in summer. The latter includes visits to typhoid infected places, infection from polluted waters, fly infection, and contact infections.

The cases that are concentrated in definite areas may be regarded as of local origin, and inquiry should be directed to the milk supply, wells, food supplies, and to social gatherings. In Rockford, the possibility of infection through the connection between the public water-supply mains and the factory fire-fighting systems was carefully considered because these systems are, in many cases, fed from Rock River



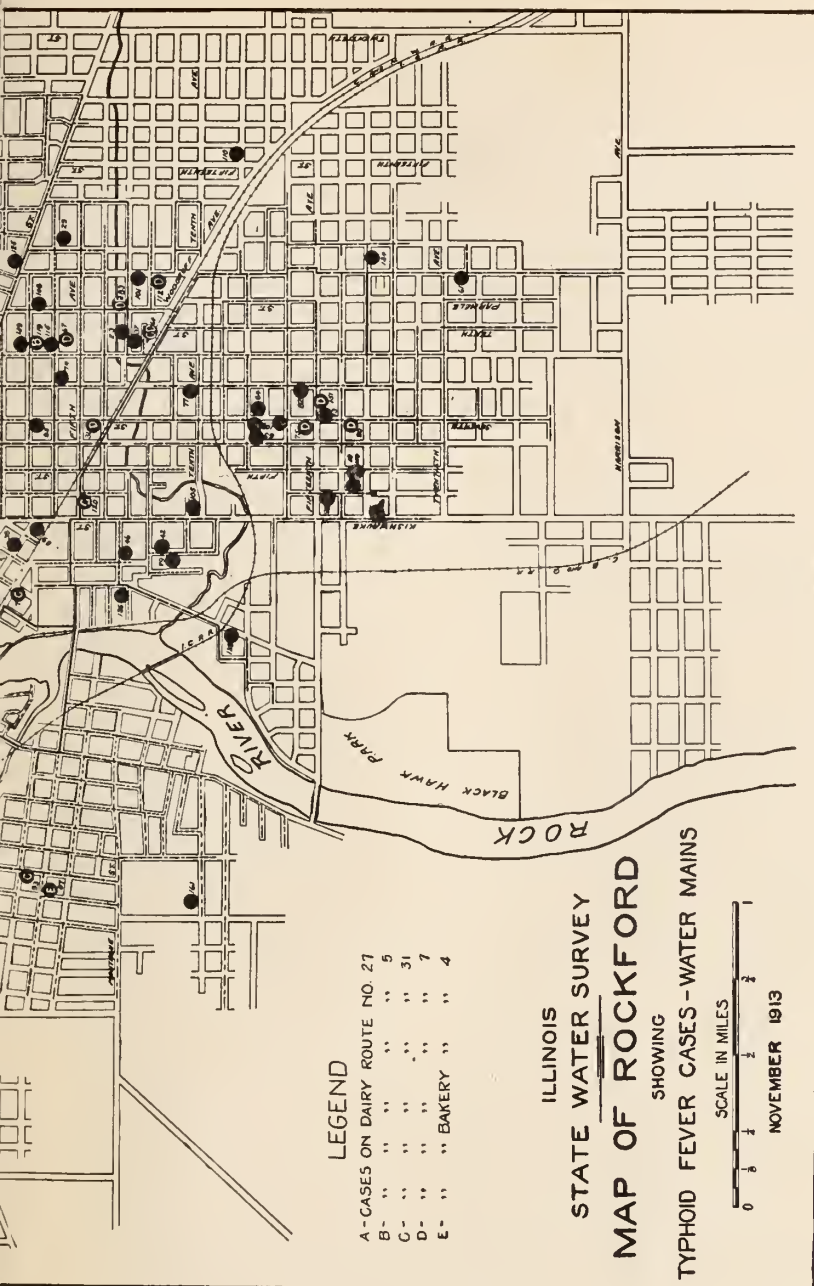


Diagram 5.

and other polluted sources. On these points, Diagram 4 gives little evidence, and so a closer analysis of data is necessary.

Distribution According to Sex and Age.—The distribution of cases according to sex and age is suggestive. Table 1 shows that the sickness was more prevalent amongst males than females, which attracts attention to those modes of infection that especially affect the male on account of his tendency to move about. This preponderance of cases in the males is in striking contrast to the water-borne epidemic of 1912, when the infection reached all alike and when the division of cases between the sexes was practically equal.

TABLE 1
DISTRIBUTION ACCORDING TO SEX AND AGE

Sex	Age								Total	Percentage
	0-9	10-19	20-29	30-39	40-49	50-59	60 and Above	Unknown		
Male	6	28	31	17	7	8	3	4	104	64
Female	5	11	19	11	5	5	1	4	61	36
Total	11	39	50	28	12	13	4	8	165	100

To show the comparison between the age distributions in the two epidemics, Table 2 is introduced.

TABLE 2
PERCENTAGE OF TOTAL NUMBER OF PATIENTS BETWEEN THE AGES INDICATED IN EPIDEMICS OF 1912 AND 1913

Age	Percentage Between 0-5 Years	Percentage Between 6-15 Years	Percentage Between 16-30 Years	Percentage Between 31-45 Years	Percentage at 46 Years and Above	Percentage of Unknown Age
1912	6.9	23.3	46.0	14.8	9.0	—
1913	3.0	17.6	44.3	18.2	12.1	4.8

It is apparent that there is not much difference, tho in 1913 there were 4.8 percent more typhoid patients above the age of sixteen than in 1912. As a matter of fact, to obtain the true age distribution, it must be studied in relation to other groups.

After this general review of the cases, an attempt will be made to throw more light on their origin by developing certain special groups.

SPECIAL GROUPS

Occupational.—The typhoid fever victims followed occupations of many sorts, and there was no concentration of cases in any single trade, nor in any one place. The greatest number of cases appeared at the Union Furniture Co.'s factory, where eight employees had the disease. It is possible that some of them may have been infected by flies from a large, insanitary privy vault, twenty-five feet square, that was used by all of the workmen. This same vault menaced the well from which drinking water was taken, and, for that reason, was closed in October by the health department, but it seems improbable that the well was actually infected, for there were only eight cases during a period of

TABLE 3
AGE DISTRIBUTION OF GROUP 2

Sex	Age							Totals
	Years 0-9	Years 10-19	Years 20-30	Years 30-39	Years 40-49	Years 50-59	60 Years and Above	
Male	2	19	10	2	3	0	1	37
Female ...	1	1	3	2	1	0	0	8
Total ..	3	20	13	4	4	0	1	45

TABLE 4
DETAILS OF DISTRIBUTION OF CASES REPRESENTED IN TABLE 3

Sex	Travelers	Bathers	Boaters	Fishers	Total of Number of Cases	Percentage of Total
Male	22	25	3	5	37	82
Female	5	2	5	0	8	18
Total	27	27	8	5	45	..

seven weeks amongst the one hundred and fifty to two hundred employees. The surroundings of this factory and of others in the same section of the city are very bad owing to the absence of sewerage. The land in the vicinity is flat and frequently flooded with water that is saturated with sewage and other filth, consequently the land becomes grossly contaminated and affords a breeding place for mosquitoes in summer. Tho some of the places are insanitary, there is no evidence to show that any of them was responsible for the outbreaks.

Travelers and Pleasure Seekers.—In Diagram 6 are displayed those cases that developed amongst those who were traveling, or who were

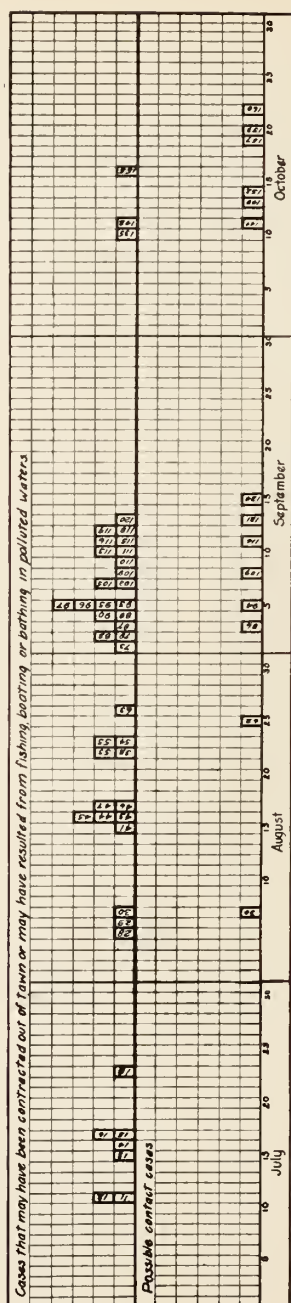


Diagram 6.—Cases among travelers and pleasure seekers, and possible contact cases.

camping, boating, bathing, or fishing on the dangerously polluted streams of the vicinity. Some discretion was exercised in making this group; the cases of people who were out of town but who probably did not contract the contagion abroad are excluded. Table 3 shows the age and sex distribution of the cases plotted on the diagram, and Table 4 shows a detailed distribution of cases represented in Table 3. It should be noted in Table 4 that some of the forty-five cases appear in more than one of the four classes of cases, thereby producing the false impression that more than forty-five cases are being considered.

Thus, it may be inferred that forty-five out of the one hundred and sixty-five patients probably were infected outside of Rockford, or as a result of bathing, boating, or fishing in polluted water. The marked preponderance of males, especially between ten and twenty-nine years, adds force to this theory. It is not contended that all of these forty-five patients had been infected necessarily in this manner; in fact, a number of cases can be otherwise accounted for. However, the figures are significant as indicating the importance of travel, vacations, and outings as agents of summer typhoid. The occurrence of so many cases amongst those seeking recreation on neighboring streams, particularly Rock River, emphasizes the necessity of keeping these streams reasonably clean. In the matter of pollution, Rockford itself is an offender; many of its sewers discharge into the river above the dam and into neighboring small water courses.

Not a few citizens held the opinion that the well water at various recreation parks near the city was responsible for many cases of typhoid and a well at the golf grounds in Sinnissippi Park was regarded with great suspicion. By reason of its inaccessibility, isolation, and construction, it is improbable that this well was a factor in the typhoid situation, nor is there epidemiologic evidence that any of the other park wells was concerned. Nevertheless, these parks, in another way, may have contributed to the typhoid cases, for, through carelessness or filthy practices in the use of some of the privies, infection may have been passed from one person to another by a sort of indirect contact. Still, except that the bathers at Love's Park and more especially at Black Hawk Park may have contracted the contagion, there is no reason for believing that the recreation parks were, at most, aught but occasional and practically negligible factors in the dissemination of typhoid fever.

Secondary Cases.—In every outbreak of typhoid, after a time equal to the incubation period of the disease has passed, secondary cases appear in some of the houses. These cases are commonly attributed to contact infection; that is, they are believed to be contracted from germs discharged in the secretions and excretions of the primary patient. The numbers of secondary cases vary markedly in different places. Thus, Jordan, while investigating the undue prevalence of typhoid in Winnipeg, Manitoba, 1900-1903, in the course of a house to house canvass of one district, by no means the worst, found secondary cases in 56 percent of the houses. In Rockford, during the present outbreak there were but eleven secondary cases, which represent about 7 percent of all the houses studied. Diagram 6 shows the comparatively unimportant part played by secondary infection. The small number of contact cases is accounted for, in part, by the rather large number of patients treated at the two hospitals; in part, by the prophylactic measures taken by the local physicians; and, in part, by the systematic disinfection of latrines by the board of health after the epidemic in 1912.

Social Gathering as an Agent.—Seven cases appear to have been contracted at a supper given a ladies' club in a private residence, on August 25. The hostess had typhoid in 1903, her husband, in 1905, and her daughter, in 1907 or 1908. This series of cases, culminating in the cases amongst the guests at this supper at which sandwiches and cold pressed meat, prepared by the hostess, were served, suggests a typhoid carrier as the source of the infection. Unfortunately, this suspicion has never been verified because samples on which to make the necessary tests were never furnished.

Group of Cases Due to Bread and to Milk Infections.—The groups of cases which have been described are of relatively minor importance to four large and inter-connected subgroups that involve one bakery and three milk-supply stations.

There is a large consumption of bakery goods in Rockford. Table 5 gives a list of bakeries, the customers of which had typhoid, the output of each, and the number of cases of typhoid fever amongst the customers of each firm. It is evident that Bakery 4, one of the smallest, had the largest number of typhoid fever cases.

The milk-supply of Rockford comes from the surrounding country and is, for the most part, distributed by contractors who purchase the

milk of farmers and bottle it in the city. The equipment of these city milk plants varies from very meager to practically complete, and the conditions in them range from poor to excellent. For several reasons, the attention of the investigators was soon directed to the milk-supply. After the farms, whereon the milk under suspicion was produced, were carefully inspected, the conclusion was reached that the milk was infected after it left the farms. This is an important point and emphasizes the need of proper handling of milk by contractors, and the necessity of rigid inspection of city milk plants by agents in the employ of the municipality or of the state.

TABLE 5
STATISTICS FROM THE INVESTIGATION OF BAKERIES

Bakery	Approximate Number of Loaves of Bread Daily	Number of Typhoid Cases Among Customers
1	650	2
2	600	7
3	2,500	18
4	350	29
5	1,500	3
6	6
7	1,000	7
8	8,000	9
9	800	5
10	2,500	14
11	1
12	600	4
13	3,000	20

Table 6 gives a list of the dairies, amongst the customers of which typhoid fever occurred; the daily output; the number of customers of each concern; the number of patrons who had typhoid fever during the period extending from July 1 to November, 1914.

Table 6 shows that there is a disproportionate number of typhoid cases amongst the customers of Dairies 5, 27, 7, and 31, for the first two of these firms furnished but 71.0 percent of the milk and had 41.2 percent of all the cases on the milk routes, and the four firms together furnished 52.5 percent of the milk and had 63.7 percent of the cases. Tables 5 and 6, then, implicate certain firms engaged in vending bread and milk in the spread of typhoid fever, but nothing more than this may be justifiably adduced from the tables. However, by a study of the map, Diagram 5, which gives the geographical distribution of cases in the city, and by an analysis of Diagram 7, which shows the chronological distribution of cases occurring on the routes of the firms under

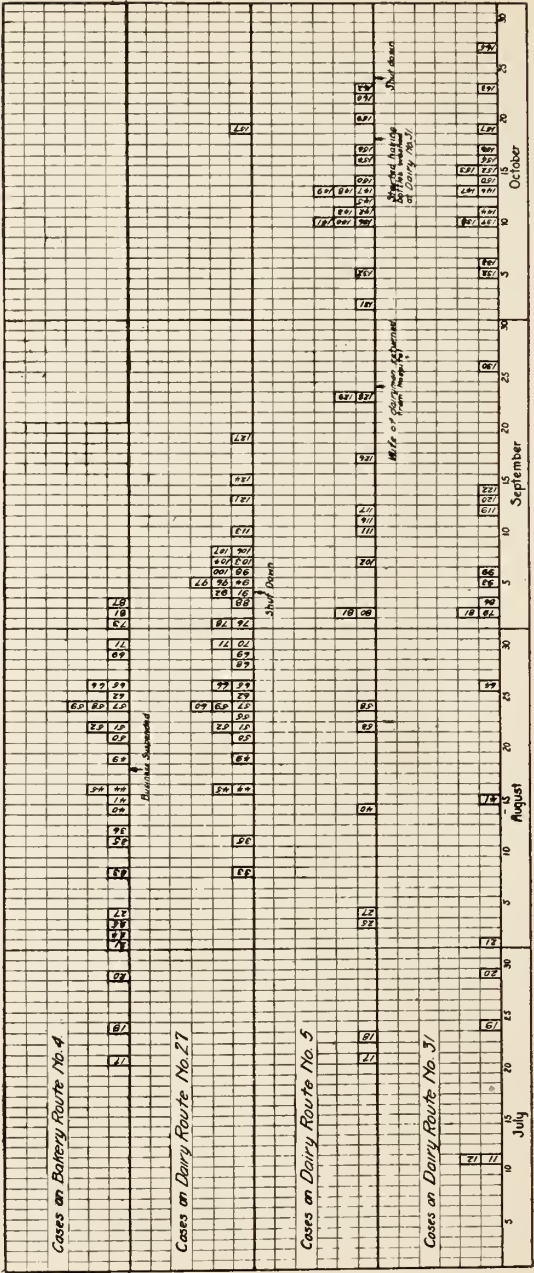


Diagram 7.—Chronological distribution of cases occurring on the routes of four firms.

discussion, and by the application of facts gleaned in the course of a house to house canvass, a correct idea may be had of the way these firms involved each other and spread contagion in those parts of the city where they operated.

TABLE 6
STATISTICS FROM INVESTIGATIONS OF MILK ROUTES

Dairy	Quarts Daily	Number of Customers	Number of Cases of Typhoid Fever on Route
1	250	200	2
2	300	223	2
3	300	150	5
4	210	170	1
5	500	450	33
6	300	200	2
7	400	175	8
8	2
9	180	160	3
10	400	340	3
11	160	95	3
12	250	160	1
13	250	200	2
14	2
15	1
16	550	250	5
17	1
18	*	...	3
19	175	150	1
20	325	200	2
21	270	225	2
22	1
23	1
24	180	150	2
25	200	...	1
26	260	150	2
27	380	350	38
28	1,300	1,100	8
29	*	...	1
30	200	...	2
31	5,300	5,600	31
32	200	1

* Only a few quarts to neighbors.

Diagram 5 shows that the cases on the routes of the dealers under suspicion are localized in the northwestern part of the city, in the section between the forks of Kent Creek. All of the cases on the route of Dairy 27 were here because the route did not extend farther. There was a sprinkling of cases from Dairy 5 between the north branch of Kent Creek and the Rock River marking the more extended route of the dairy. Cases from Dairy 31 and Bakery 4 were sparsely scattered over the greater part of the city, since these concerns deliver over a wide area. The chief value of the map is to show the territory covered by the several firms and that since they operated in the same circuit, to a considerable extent, they had the same patrons which afforded the firms ample opportunity to infect each other.

Bakery 4.—The cases were few and scattered during the latter part of July; increased in number during the first part of August; became of almost daily occurrence after August 10, and ended abruptly on September 3, because, on August 18 the business was suspended by order of the local health department. Inquiry revealed the fact that Case 25 was a man who was employed in the bakery as a driver of the delivery wagon. He usually worked from 3 until 5 o'clock in the mornings, and occasionally helped to wrap the loaves of bread. For over a month this man kept at work, altho he felt badly and suffered from diarrhea. About ten days before he stopped work at the bakery his case was diagnosed by a physician as typhoid fever, but he did not give up and take to his bed until August 3, the date at which his case appears on the diagram. There were twenty-nine cases of typhoid fever amongst the customers of the bakery; of these cases fifteen occurred three weeks after the driver gave up, and five within two weeks before he gave up. Furthermore, there are displayed on the diagram twenty-three cases which occurred within this same period on the routes of Dairies 5, 27, and 31, and of these twenty, or 87 percent, were customers of Bakery 4. Sufficient evidence to explain all of these cases seems to have been found in this one patient, Case 25, for they all fall within the limits of the period of infectivity, and the work of Katharine Howell² shows that the intestinal germs may be carried on bread, and her work is confirmed by a bread-borne epidemic of typhoid fever at the Government Hospital for the Insane, at Washington, D. C. However, investigation of the milk routes shows that it is not necessary, or perhaps even reasonable, to believe that this was the sole means of infection.

Dairy 27.—The conclusions about the relation of the milk routes to the epidemic were reached after careful inquiries at the homes of the patients and after testing the various possibilities that the histories suggested.

Cases began to appear on this route after the first week in August and, by the end of the second week, were so numerous as to plainly mark an epidemic. About the same number of cases continued to be reported daily through the first third of September, after which they dwindled down because the dairy was closed by order of the local health department on September 3.

From August 8 to September 19, thirty-eight cases of typhoid appeared on the route. Of these, thirteen, or 34.2 percent, fall into the Bakery 4 group: five, or 13.1 percent, in the group of travelers and pleasure seekers: three, or 7.9 percent, in the group of secondary infection; seventeen, or 44.7 percent, find explanation only in milk infection.

Two cases were obviously due to milk infection. Case 106 was a little girl who lived in a remote part of the city but who drank milk from Dairy 27 while visiting her cousin; no other clue to the mode of infection was found. Case 107 was a child, 2 years of age, who drank well-water and milk from Dairy 27. As no suspicion was attached to the water and as the child had not been off the premises within the incubation period, the milk was held accountable for the sickness. Diagram 7 shows that eight cases of typhoid fever appeared on the route of Bakery 4 more than twenty-one days after the driver, Case 25, had stopped work. It is barely possible that some of these cases may have had long prodromal periods, but it is more plausible to regard them, and perhaps even some of the cases that preceded them, as having another origin. Of these eight cases, five were on the route of Dairy 27 and one on Dairy 5. These cases suggest milk infection, and there are several ways in which milk from Dairy 27 may have been infected.

This route may have been infected through the agency of milk bottles, especially as they were not sterilized. Any one of the early typhoid-stricken customers of Bakery 4 may have started the trouble for Dairy 27 by returning infected milk bottles, and later the milk route may have been repeatedly reinfected in this manner not only from the sick customers of Bakery 4, but from customers of Dairy 27 who had typhoid and who were returning milk bottles. Against this it may be urged that for eighteen days after patrons of Bakery 4 began to take to their beds, the route of Dairy 27 remained free from typhoid. Moreover, not every milk bottle that is taken into a home harboring a communicable disease becomes infected. On the contrary, it is necessary for the bottle to be handled by the patient, or by one of the patient's attendants, if it is to become a vehicle for the transmission of the disease. Arguments against this mode of infection of Dairy 27's route is to be found in the history of Case 49.

Another possible mode of infection of the route of Dairy 27 is direct contact infection. Case 49 was a girl, seven and one-half years old, who ate cakes and bread from Bakery 4, and who, according to all accounts, was not well for some time before her sickness was diagnosed as typhoid fever. She was put to bed on August 19. The patient lived in a house adjoining that of the proprietor of Dairy 27. He admitted that the little girl had been allowed to come in the milk-house and had, at times, helped to cap the milk bottles, consequently the caps may have been infected by her fingers. The child would naturally handle any objects within her reach, and so it is not unlikely that other things, such as, bottles, pails, can covers, strainers, etc., may have been also infected. Then, again, the child may have been the cause of indirect infection of the milk, as the brother of the little girl worked for Dairy 27 as driver, in which capacity he not only delivered the bottled milk but collected the milk in cans from farmers. The brother may have carried typhoid germs from his sick sister to the milk, altho he did not develop the disease himself. Except in the prodromal stage of the disease, the boy and girl seem to have been separated. This weakens the probability of the boy having infected the milk in this way, yet there is another chance for the milk of Dairy 27 to have been infected by a temporary carrier, for when the local health department stopped this boy from working for this dairy he was succeeded by a boy in whose household was Case 32. As it was reported that empty milk bottles were picked up from doorsteps on the trip to the farm for milk and were filled by the boys on the way back by dipping the bottles into the cans, the milk may have been infected in this manner. However, infection of the milk by temporary carriers, if it actually occurred, serves rather to explain how the infection was prolonged on this route than to account for the primary infection of the route.

There is one more factor which enters into this: The milk house of Dairy 27 swarmed with flies, and was next to the home of Case 49. Still, this mode of infection seems remote, inasmuch as there was no privy on the premises.

It is believed to be unfruitful to speculate as to which of these possible modes of transmission was actually the most potent. Probably the infection was started in Bakery 4 and was handed about in all the ways enumerated.

Dairy 5.—This dairy is superior to the preceding one. The milk comes from clean, prosperous farms, and the milk house is kept in good order, but it is not equipped with apparatus for sterilizing bottles, cans, and other utensils.

Beginning July 21, typhoid fever began to appear on this route, but there were only a few cases through August. Early in September the frequency of cases attracted attention. The number dropped off coincidently with the falling

off of the cases on the route of Dairy 27 only to increase rapidly from September 10. As cases continued to break out on the route, the local board of health, on October 18, fearing that the bottles from Dairy 5 might have become infected, required that they be disinfected daily at the plant of Dairy 31. Five days later, the board closed down the route. Of the thirty-three cases that occurred on the route of Dairy 5 between July 1 and November 1, six, or 18.2 percent may be attributed to bread from Bakery 4; five, or 15.1 percent, of the patients had been travelers and pleasure seekers; three, or 9.1 percent, fall in the group of secondary infection; for nineteen, or 57.6 percent, the only explanation is milk infection.

Of the seven cases between July 21 and August 24, on the route, six were users of bread from Bakery 4, and they came down within the period beginning two weeks before and ending three weeks after the driver (Case 25) took to bed; the other case falls in the group of travelers and pleasure seekers. Therefore, it is not impossible that the route of Dairy 5 remained uninfected till September 1. However, there were other ways in which the milk may have been infected, and these must be considered.

Either of two people may have infected the route. The first infection may have come from the sick employee at Bakery 4, Case 25, for he was in the habit of helping himself to milk by dipping it out of the cans in which it was daily left at the bakery. These cans were returned and were used again without sterilization. Case 40, wife of the proprietor of Dairy 5, may have infected the milk. She may have contracted the disease from goods from Bakery 4, which she regularly used, or from milk from Dairy 5 if it had been infected by Case 25, and, in ways unknown, she may have communicated her own infection to the milk prior to her removal to a hospital. That, after her return on September 24, she did infect the milk seems to be a fact, for sixteen days thereafter typhoid fever again flared up on the route of Dairy 5, and before the route was shut down, on October 24, thirteen cases, or 44.8 percent of all the cases on the route in the interval from July 1 to October 24, were recorded.

It was found that Dairy 5 did not knowingly take milk bottles from places where there was communicable disease, and that bottles of other dealers were never used. Nevertheless, this route may have been infected from infected milk bottles, either bottles collected from customers before the dairy had been warned that they had the disease, or much more likely their own bottles which were collected and used by Dairy 27, and which, before reaching Dairy 5 again, may have been infected in the homes of patients on the route of Dairy 27, or in the course of handling by the little girl, Case 49, or by the driver employed by Dairy 27.

Dairy 31.—This dairy has a thoroughly equipped, modern, city milk plant, and does, by far, the largest milk business in Rockford. This company has apparatus for sterilizing cans and bottles, and all the milk is pasteurized by the "held" process. At first sight, it would seem unlikely that milk from a thoroughly equipped plant could become infected, but, on the other hand, the efficiency of the machines depends on the faithfulness and intelligence with which they are operated.

The run of cases on this route was similar to that on the route of Dairy 5, but began a month later. Between July 1 and November 1, there were thirty-three cases on the route. This means 0.5 percent of all the customers, which indicates that if the route was at all infected it was so but slightly. Of these cases, four, or 12.9 percent, fall into the Bakery 4 group; seven or 22.5 percent, in the group of travelers and pleasure seekers; four, or 12.9 percent, in the group of contact infection; and sixteen, or 51.6 percent, may be explained only as milk infection.

The only mode of infection of the dairy seems to have been through the use of bottles from Dairy 5 and possibly from Dairy 27. That there was an intimate relation between these two routes is apparent from the synchronous rise and fall of cases on the two routes at the time of the recrudescence of typhoid on the route of Dairy 5 sixteen days after the return of Case 40 from the hospital, and at the time Dairy 5 was closed.

Dairy 7.—It is believed that the cases on this route were not related to the foregoing dairy cases nor to bread from Bakery 4, for the reasons that the routes did not cross each other at many points and so did not afford customers the chance of infecting this route. Of the eight cases on this route, two, or 25 percent, fall in the group of travelers and pleasure seekers, and six, or 75 percent, may be best explained by milk infection. There is no reason to believe that Cases 13 and 31 (Diagram 8) were infected by this milk from this dairy, but the subsequent cases may have been caused by infected bottles from Case 31. Case 75 is interesting. The patient became a mother just one month before being attacked with typhoid, and the good care which she received at the time makes any other source of the disease than her food improbable. The only food which she had that was under suspicion was milk from Dairy 7.

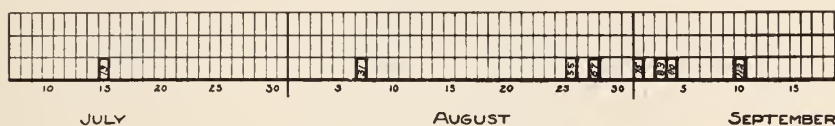


Diagram 8.—Cases on route of Dairy 7.

Grouping of Cases with Reference to Drinking Water.—The care with which the water supply was safeguarded following the water-borne epidemic of 1912 has already been outlined. The only really vulnerable components of the system at the time of the outbreak were the various connections with the factory fire protection systems that take water from the contaminated Rock River. The danger here is reduced to a minimum by the use of double check valves, together with means for readily testing their condition, and, further, by systematic monthly inspection of all these connections. At one factory, on three different occasions, both check valves were found open, so that it must be admitted that they do not afford complete protection. Since these valves would be opened only in the event of being leaky, or of heavy flushing of the watermains, or of severe fires, the records of the inspector, A. C. Lyons, and those of the water works and of the fire department were carefully studied to see whether or not anything could be discovered to indicate that an opening of the valves actually did take place. Briefly, it was found that there was only one factory fire protection system that was so located that it could supply water to the district between the forks of Kent Creek, where most of the typhoid cases occurred, and, moreover, the records show that during 1913 this

particular system was never under fire pressure and was filled only with city water. There were no flushing operations of sufficient magnitude to open the double check valves. There remains only the question whether there were fires big enough to have caused sufficient draft of water to have opened the valves.

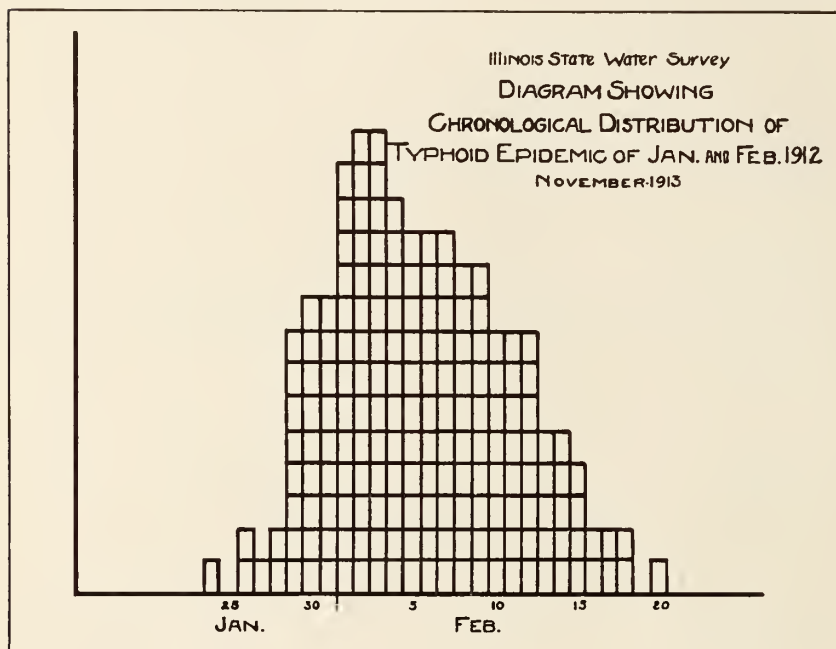


Diagram 9.

Considering the incidence of cases, it is difficult to place the probable time when the water may have been infected, for this outbreak is wholly unlike a water epidemic. By comparing Diagram 4 with Diagram 9 it will be seen how, in 1913, the cases strung along, whereas in 1912, a typical water outbreak, one can determine almost to a day when the infection took place. To account for the typhoid outbreak in 1913 one must either assume three separate infections, in the middle of July, in August, and in September, or else assume an infection in the middle of July and prosodemic infection thereafter. As there was not a conflagration at any of the times indicated, this explanation fails.

An approach from another angle also leads to the conclusion that the water was not a factor in the typhoid fever situation. Of the one hundred and sixty-five cases investigated, one hundred and six, or

64 percent, used city water only; thirty, or 18 percent, used well water only, and 12 percent used both city and well water, leaving ten cases, or 6 percent, in which the drinking water is unknown. That so large a proportion of the sick did not use city water casts doubt on the supposition that water was the source of infection, and in any event eliminates it as the sole cause of infection. The case against the city water appears still weaker when it is known that twenty of the cases reported as using only city water fall in the group of travelers and pleasure seekers. This leaves seventy to eighty cases scattered throughout the city that may or may not have been due to water infection. Of these, all but about a score of cases on the east side can be explained on the basis of bread and milk infections. Were the facts as to these twenty cases more complete, different explanations of how they were contracted might perhaps be given, but, as it is, these twenty cases can be regarded only as having arisen from a variety of insanitary conditions forming part of the environment.

Private wells were, in no specific instance, suspected of spreading typhoid, but it is felt that some of the obscure cases may have had such an origin.

CONCLUSIONS

The outbreak did not originate in a single source of infection but was derived from several sources, giving rise to several distinct groups of cases that remained isolated or that fused and produced complicated inter-relations.

The largest group comprised ninety-five cases, all of which were pretty certainly traced to an employee of Bakery 4. Through him, a number of people were infected by contaminated bread, or pastry, or both, and through one or more of these people, in turn, Dairies 5, 27, and 31 were involved. Infected milk bottles were the principal means of spreading the disease, tho other more or less obscure agents were operative.

The public water supply was ~~not~~ ⁽³⁾ responsible for the 1912 outbreak. ?

One small group of cases resulted from a social gathering under conditions that suggested a carrier.

There was a marked number of cases amongst vacationists and others who fished, boated, and bathed in neighboring, polluted streams.

There were some cases of obscure origin that may be attributed to shallow wells, privies, fly infection, and various insanitary conditions.

Secondary cases were rare.

THE EFFECT OF QUININ ON RABIES *

CHARLES KRUMWIEDE, JR., AND ALICE G. MANN

(From the Research Laboratory, Department of Health, New York City)

About a year ago, Moon¹ reported the recovery of three dogs treated with quinin after the development of symptoms of rabies. We attempted to repeat his work, but because of the results obtained the work was discontinued. The recently reported, negative results of Cummings,² who found that quinin had no prophylactic nor curative effect in rabbits, and the equally negative results of Frothingham and Halliday,³ using rabbits, seem to warrant the addition of our results.

A series of rabbits were inoculated with fixed virus through a trephine opening, and the injection of quinin was started on different days thereafter. The quinin was the bisulphid, and a 1-8 solution in water was used. The inoculations were made August 9, and Rabbits 1 and 2, which received no quinin, became paralyzed August 15, and died August 17. Rabbits 3, 4, and 5, which received 2 c.c. of the quinin solution on August 14, as well as Rabbits 6 and 7, which received 2 c.c. of quinin solution on each day from August 11-15, also became paralyzed on August 15, and died August 17. The remaining rabbits in the series were killed by the quinin; 12 c.c. killing in a few hours, 8 c.c. over night, and 4 c.c. after several doses.

No difference could be noticed in any of the rabbits. The treatment was started so early that it would be expected to manifest some influence if it possessed any potency.

Six dogs were then inoculated with rabies and treatment with quinin was started on a companion dog as soon as any symptom developed in a dog of similar weight. The dogs were inoculated intracranially with street virus on August 9.

Dog 1, a pup, became depressed August 19; paralyzed August 20; died the next day.

Dog 2, a black and tan, weighing 7 pounds, showed no symptoms on August 21 and received 15 gr. of quinin sulphate, in capsules of 5 gr., every day from August 21 to September 2, when it appeared well and lively; September 12, it

* Received for publication September 3, 1914.

1. Jour. Infect. Dis., 1913, 13, p. 165.

2. Ibid., 1914, 15, p. 205.

3. Jour. Med. Research, 1914, 30, p. 275.

showed weakness of the hind legs; September 13, weakness and a high-pitched bark; September 15, death.

Dog 3, 24.5 pounds, showed weakness August 24; became paralyzed August 25; died the next day.

Dog 4, weighing 16.25 pounds, showed no symptoms on August 25 and was given 20 gr. of quinin sulphate in capsules of 5 gr.; August 26, it received 12 c.c. of a solution quinin bisulphate 1-8; August 28, there was spastic paralysis, and it received 10 gr. of quinin sulphate and 16 c.c. of the bisulphate solution; on August 29 it received 15 gr. of the sulphate and 16 c.c. of the solution; August 30, died.

Dog 5, terrier, 18.25 pounds, appeared excited on August 25 and received 20 gr. quinin sulphate; August 26, it received 5 gr. of quinin sulphate; August 27, 10 c.c.; August 28, 16 c.c.; died August 29.

Dog 6, mongrel, 18 pounds, acted suspiciously August 24; August 25, received 25 gr. of the sulphate, it was excited and rather weak; on August 26 it received 30 gr.; August 27, 40 gr.; August 28, 25 gr.; August 29, it seemed somewhat improved and received 30 gr.; again on August 30 and 31; on September 1 its condition was good, altho there was a slight weakness, and it vomited about half of the quinin given that day, namely 30 gr.; found dead, September 2.

In all dogs, the brain contained typical Negri bodies, and material from each dog produced rabies in guinea-pigs, indicating that the virulence was not destroyed by the treatment.

The only possible effect of quinin in these experiments is the remission in Dog 6 and the prolongation of the period of incubation in Dog 2.

SUMMARY

Of four dogs treated with quinin, one showed a prolonged period of incubation and another showed a remission in the course of the disease, in either case, however, not beyond variations in the natural course of the disease. The fact that these variations occurred in the two of four dogs receiving the largest amounts of quinin is probably only a coincidence. No influence was observed on the period of incubation in rabbits.

ON THE RELATIVE VIRULENCE OF SENSITIZED AND NON-SENSITIZED TYPHOID BACILLI *

RUSSELL L. CECIL

(From the Medical Clinic of the Presbyterian Hospital, New York City)

In 1910 Garbat and Meyer¹ published the results of an experimental study of a typhoid immune serum produced in rabbits by repeated injections of typhoid bacilli. These investigators found that rabbits, injected intravenously with killed sensitized typhoid bacilli, developed a serum of higher protective power than the rabbits injected with killed, non-sensitized bacilli. Furthermore, they noticed that the animals endured the sensitized better than the non-sensitized bacilli and recovered more quickly. These investigators were concerned chiefly with the relative power of the serum produced by the injection of sensitized and non-sensitized typhoid bacilli; the question of the relative virulence of the bacilli was only casually touched upon. Ficker² has pointed out that the two antigens, as prepared by Garbat and Meyer, are not comparable, since the sensitized bacilli were washed and the non-sensitized unwashed.

Besredka³ found that the sensitized vaccine of typhoid and cholera (killed by heating one hour at 60 C.), when injected intraperitoneally in guinea-pigs, was less toxic than the unsensitized vaccine. Besredka has also shown that subcutaneously the living, sensitized typhoid bacilli are much better tolerated than killed, non-sensitized ones. In view of this fact and the superior immunizing properties of the sensitized vaccine, Besredka has recommended its use in practice instead of the usual vaccine of Wright.

While there seems to be no doubt of this difference in virulence when the bacilli are injected subcutaneously or intraperitoneally, the evidence in the case of intravenous injection is not conclusive. The present experiments were undertaken to settle this point. In view of our recent knowledge of anaphylaxis, it was hardly to be supposed that sensitized typhoid bacilli, injected intravenously, would be less virulent than non-sensitized bacilli. Indeed, the contrary might have been

* Received for publication September 22, 1914.

1. Ztschr. f. exper. Path. u. Therap., 1910, 8, p. 1.

2. Handbuch der Pathogenen Mikroorganismen, 1913, 2, p. 172.

3. Ann. de l'Inst. Pasteur, 1902, 16, p. 918.

expected. My experiments, however, support the observation of Garbat and Meyer, namely, that sensitized typhoid bacilli, when injected intravenously, have less virulence than non-sensitized typhoid bacilli.

The same technic was employed throughout the experiments.

A number of agar slants were inoculated with a stock strain of the bacillus typhosus. After 24 hours' incubation at 37 C. the growth was washed off with normal salt solution, and the clumps were broken up by thorough shaking. In the experiments where killed bacteria were used, the next step was to heat the suspension of bacilli in a water bath for one hour at 60 C. The suspension of bacteria (either killed or living) was now divided into two equal parts. To one part were added 5-10 c.c. of inactivated antityphoid rabbit serum; to the other part, an equal quantity of salt solution. The two suspensions were then placed in the water bath at 37 C. for one hour, and then in the icebox for another hour. The sensitized portion now showed marked agglutination. The suspensions were then centrifuged until both were absolutely clear, the supernatant fluid was pipetted off, and the sediment was washed twice with normal saline solution. Each sediment was next rubbed up with a small quantity of salt solution until perfectly homogeneous and then made up to the desired amount with the same solution. The suspensions were not allowed to stand long; they were almost immediately injected into animals.

The typhoid immune serum was obtained from rabbits which had received a number of intravenous injections of typhoid bacilli, first killed bacilli, then living. The serum was always inactivated by heating for one-half hour at 56 C.

Before proceeding to the experiments proper, it seemed desirable to determine the protective power of the antityphoid serum which was to be used. Increasing amounts of a twenty-four-hour broth culture of the bacillus typhosus (homologous strain) were injected intraperitoneally in white mice, while an equal number of mice received corresponding amounts of culture freshly mixed with typhoid immune serum (agglutinating titer = 1-4,000).

Mice Receiving B. Typhosus			Mice Receiving B. Typhosus + 0.2 c.c. of Typhoid Immune Serum		
1. Broth culture 0.5	c.c.	Died.	1. Cultures 0.5	c.c.	Died.
2. Broth culture 0.1	c.c.	Died.	2. Cultures 0.1	c.c.	
3. Broth culture 0.01	c.c.	Died.	3. Cultures 0.01	c.c.	
4. Broth culture 0.001	c.c.	Died.	4. Cultures 0.001	c.c.	
5. Broth culture 0.0001	c.c.		5. Cultures 0.0001	c.c.	
6. Broth culture 0.00001	c.c.		6. Cultures 0.00001	c.c.	

It will be seen from the protocol that the serum protected in mice against 100 times the lethal dose of typhoid bacilli.

Experiment 1.—To determine the relative virulence of living, sensitized and non-sensitized typhoid bacilli, Experiment 1 was made.

Suspensions were made up so that 1 c.c. equaled one-half slant agar of the bacillus typhosus. The results with rabbits injected with sensitized typhoid bacilli were as follows:

- Rabbit 1, 1,420 gm. 0.1 slant, intravenously.
- Rabbit 2, 1,400 gm. 0.25 slant, intravenously.
- Rabbit 3, 1,380 gm. 0.5 slant, intravenously.
- Rabbit 4, 1,350 gm. 0.75 slant, intravenously.
- Rabbit 5, 1,320 gm. 1.0 slant, intravenously.

Rabbit 6, 1,260 gm. 1.5 slants, intravenously.

Rabbit 7, 1,220 gm. 2.0 slants, intravenously.

Rabbit 8, 1,220 gm. 2.5 slants, intravenously. Died night after injection.

Rabbit 4 died of coryza six days after injection. Culture from heart's blood gave pure growth of an influenza-like bacillus.

Rabbit 7 died ten days after injection. Culture from heart's blood gave pure growth of streptococcus.

Culture from the heart's blood of Rabbit 8 gave pure growth of *B. typhosus*.

The results with the rabbits injected with non-sensitized typhoid bacilli are as follows:

Rabbit 1, 1,460 gm. 0.1 slant, intravenously.

Rabbit 2, 1,420 gm. 0.25 slant, intravenously.

Rabbit 3, 1,400 gm. 0.5 slant, intravenously.

Rabbit 4, 1,320 gm. 0.75 slant, intravenously. Died night after injection.

Rabbit 5, 1,240 gm. 1.0 slant, intravenously. Died night after injection.

Rabbit 6, 1,240 gm. 1.5 slant, intravenously. Sick, but recovered.

Rabbit 7, 1,160 gm. 2.0 slant, intravenously. Died day after injection.

Rabbit 8, 1,150 gm. 2.5 slant, intravenously. Died night after injection.

Cultures from heart's blood of Rabbits 4, 5, 7, and 8 gave growths of *B. typhosus*.

The results with the guinea-pigs injected with sensitized bacilli are as follows:

Guinea-Pig 1, 285 gm. 0.01 slant, intravenously.

Guinea-Pig 2, 270 gm. 0.02 slant, intravenously.

Guinea-Pig 3, 255 gm. 0.05 slant, intravenously.

Guinea-Pig 4, 250 gm. 0.1 slant, intravenously.

Guinea-Pig 5, 232 gm. 0.2 slant, intravenously.

Guinea-Pig 6, 260 gm. 0.4 slant, intravenously.

Guinea-Pig 7, 240 gm. 0.8 slant, intravenously. Died night after injection.

Guinea-Pig 8, 245 gm. 1.2 slant, intravenously. Died night after injection.

Guinea-Pig 9, 230 gm. 1.6 slant, intravenously. Died night after injection.

The results with the guinea-pigs injected with non-sensitized bacilli are as follows:

Guinea-pig 1, 272 gm. 0.01 slant, intravenously.

Guinea-pig 2, 262 gm. 0.02 slant, intravenously.

Guinea-pig 3, 237 gm. 0.05 slant, intravenously.

Guinea-pig 4, 247 gm. 0.1 slant, intravenously.

Guinea-pig 5, 220 gm. 0.2 slant, intravenously. Died day after injection.

Guinea-pig 6, 280 gm. 0.4 slant, intravenously. Died night after injection.

Guinea-pig 7, 272 gm. 0.8 slant, intravenously. Died 4 days after injection.

Guinea-pig 8, 255 gm. 1.2 slant, intravenously. Ill but recovered.

Guinea-pig 9, 240 gm. 1.6 slant, intravenously. Died night after injection.

Cultures from hearts' blood of all the guinea-pigs that died gave *B. typhosus*.

It will be seen from these experiments that the sensitized vaccine in both rabbits and guinea-pigs, when injected intravenously, is about one third as virulent as the non-sensitized. Two of the rabbits in the sensitized series died from other infections a number of days after the experiment. As typhoid bacilli were not recovered from their blood, there is no reason to suppose that the deaths were due directly to the sensitized virus.

Experiment 2.—To determine the relative virulence of killed, sensitized and non-sensitized typhoid bacilli, Experiment 2 was made.

The results with the rabbits injected with sensitized bacilli are as follows:

Rabbit 1, 1,400 gm. 0.1 slant, intravenously.
Rabbit 2, 1,540 gm. 0.25 slant, intravenously.
Rabbit 3, 1,600 gm. 0.5 slant, intravenously.
Rabbit 4, 1,500 gm. 1.0 slant, intravenously.
Rabbit 5, 1,410 gm. 1.5 slant, intravenously.
Rabbit 6, 1,150 gm. 2.0 slant, intravenously. Died 2 hours after injection.

The results with the rabbits injected with non-sensitized bacilli are as follows:

Rabbit 1, 1,350 gm. 0.1 slant, intravenously.
Rabbit 2, 1,500 gm. 0.25 slant, intravenously.
Rabbit 3, 1,620 gm. 0.5 slant, intravenously.
Rabbit 4, 1,530 gm. 1.0 slant, intravenously. Died 36 hours after injection.
Rabbit 5, 1,300 gm. 1.5 slant, intravenously. Died few hours after injection.
Rabbit 6, 1,160 gm. 2.0 slant, intravenously. Died day after injection.
Cultures from hearts' blood of rabbits that died were sterile.

The results with the guinea-pigs injected with sensitized bacilli are as follows:

Guinea-pig 1, 260 gm. 0.2 slant, intravenously.
Guinea-pig 2, 247 gm. 0.4 slant, intravenously.
Guinea-pig 3, 245 gm. 0.8 slant, intravenously.
Guinea-pig 4, 225 gm. 1.2 slant, intravenously.
Guinea-pig 5, 215 gm. 1.4 slant, intravenously.
Guinea-pig 6, 233 gm. 1.8 slant, intravenously. Died night after injection.

The results with guinea-pigs injected with non-sensitized bacilli are as follows:

Guinea-pig 1, 270 gm. 0.2 slant, intravenously.
Guinea-pig 2, 245 gm. 0.4 slant, intravenously.
Guinea-pig 3, 240 gm. 0.8 slant, intravenously. Died night after injection.
Guinea-pig 4, 225 gm. 1.2 slant, intravenously. Died night after injection.
Guinea-pig 5, 220 gm. 1.4 slant, intravenously. Ill but recovered.
Guinea-pig 6, 233 gm. 1.8 slant, intravenously. Died night after injection.
Cultures from hearts' blood of guinea-pigs that died were sterile, except one that showed a hay bacillus, apparently a contamination.

These two experiments show that, in the case of typhoid bacilli killed by heating for one hour at 60 C., the sensitized virus, when injected intravenously, is less toxic than the non-sensitized in both rabbits and guinea-pigs. The lethal dose of the sensitized bacteria is two or three times larger than that of the non-sensitized.

It is not at once evident why there should be this difference in virulence between sensitized and non-sensitized bacteria. Besredka⁴ has studied the local reactions produced by the subcutaneous injection of typhoid bacilli, and has found that phagocytosis is much more active with sensitized than with non-sensitized bacteria. It is possible that a similar difference as regards phagocytosis is present when the bacteria

4. Virchows Arch. f. path. Anat., 1913, 213, p. 244.

are injected intravenously and that bacteriolysis is also hastened when the bacilli are first subjected to the action of an immune serum.

CONCLUSIONS

Sensitized, living typhoid bacilli, when injected intravenously in rabbits and guinea-pigs, are less virulent than non-sensitized, living typhoid bacilli.

Sensitized typhoid bacilli, killed by heat, are in a similar way less virulent than non-sensitized, killed typhoid bacilli.

The most probable explanation for this difference is that sensitized typhoid bacilli undergo phagocytosis and bacteriolysis more rapidly than the non-sensitized bacilli.

CLASSIFICATION OF THE BACILLUS WELCHII GROUP OF BACTERIA *

J. P. SIMONDS

(From the Laboratory of Preventive Medicine, Harvard Medical School, and the Laboratory of Bacteriology of the University of Texas)

The name, *B. welchii*, represents a fairly well-differentiated group of organisms which have the following characteristics in common: They are large, anthrax-like bacilli with slightly rounded ends, non-motile, and gram-positive; spore formation is not constant in artificial media, and occurs only in neutral or alkaline media in the absence of fermentable sugar; they cause stormy fermentation of milk with the production of butyric acid, and may or may not slowly liquefy plain or sugar-free gelatin; they ferment, with production of acid and gas, all of the monosaccharids and disaccharids.

The group is thus differentiated, on the one hand, from the motile butyric acid bacilli, such as the bacillus amylobacter of Gruber and of Bredemann, which is motile, forms spores in milk, and contains granules which stain blue with iodine; and, on the other hand, from the so-called "putrefactive butyric acid bacilli," such as the bacillus chauvei, which do not cause stormy fermentation of milk, but form spores readily in most media.

Numerous strains of bacteria identical with, or closely related to, the bacillus welchii, under a number of names, have been described by different writers. At least eight diverse names have been applied: The bacillus of acute articular rheumatism (Achalme,¹ 1891); *B. aerogenes capsulatus* (Welch and Nuttall,² 1892); *B. phlegmonis emphysematosae* (Fraenkel,³ 1893); *B. enteritidis sporogenes* (Klein,⁴ 1895); *Bacillus perfringens* (Veillon and Zuber,⁵ 1898); *B. vaginae emphysematosae* (Lindenthal,⁶ 1897); *B. cadaveris butyricus* (Buday,⁷ 1898); *Granulo-bacillus saccharobutyricus liquefaciens immobilis* (Schattenfrohn and Grassberger,⁸ 1899).

While all of these organisms evidently belong to the same group of bacteria, they show certain minor differences among themselves. Heretofore, no systematic attempt has been made to classify the organisms within the bacillus

* Received for publication September 22, 1914.

1. Compt. rend. Soc. de biol., 1891, 43, p. 651.
2. Johns Hopkins Hosp. Bull., 1892, 3, p. 81.
3. Ueber Gasphegmone, Hamburg and Leipzig, 1893.
4. Centralbl. f. Bakteriologie, 1895, 18, p. 737.
5. Arch. de méd. expér. et d'anat. path., 1898, 10, p. 517.
6. Wien. klin. Wchnschr., 1897, 10, p. 3.
7. Centralbl. f. Bakteriologie, 1898, 24, p. 369.
8. Ibid., Abt. 2, 1899, 5, pp. 209, 697; Arch. f. Hyg., 1900, 37, p. 54.

welchii group. In other studies on this group, it was found both convenient and desirable to attempt to separate the various strains into subgroups. But before presenting the results of this work, it seems advisable to present briefly the conclusions reached by others.

Fraenkel⁹ and Klein¹⁰ recognized only one variety of the organisms isolated by them, namely, the pathogenic. Klein considered the non-pathogenic form identical with *B. butyricus* of Botkin.¹¹ Schattenfroh and Grassberger¹² and Passini¹³ differentiated two species, virulent and non-virulent.

Hitschmann and Lindenthal¹⁴ were unable, either from the behavior in the animal body or in cultures, to differentiate varieties. They found tests of pathogenicity especially unreliable as a means of differentiation. Passini,¹⁵ Werner¹⁶ and Rocchi¹⁷ attempted to distinguish subvarieties by means of serological reactions, but without success. Herter¹⁸ believed that there are subvarieties of *B. welchii* "based mainly on differences respecting the difficulty of sporulation, upon pathogenic qualities, hemolytic properties, indol production, rapidity of gas formation in man and animals, etc." He reported no results of any attempt to apply these factors as a basis of classification. Rosenthal¹⁹ differentiated two varieties—"variete rheumatismale" and "variete banale." His basis of classification, however, is not entirely convincing.

Jackson²⁰ described two types of the bacillus welchii. Type "A" was non-motile, produced 26 percent gas in raffinose broth and 10 percent gas in mannite broth, the reaction remaining neutral in each case, and produced 92 percent gas in lactose bile broth. Type "B" was motile, produced 22 percent gas in raffinose broth, the reaction becoming acid, and 56 percent gas in mannite broth, the reaction remaining neutral, and produced no gas in lactose bile broth.

Attempts to subdivide this group must be made only under the most exactly uniform conditions. Slight differences in conditions will disclose what appears to be a disconcerting tendency on the part of these organisms to display remarkable variations in the manner of growth and the degree and vigor of activity. For instance, depending upon the amount of free oxygen present in milk, one may find, after 24-48 hours' incubation, either no change in the medium, or coagulation without gas formation, coagulation with very slight gas formation, or typical stormy fermentation even to the extent of blowing out the cotton plug in the tube.

The present attempt at classification is based upon a study of some fifty strains isolated from various sources, the most scrupulous care being exercised to obtain pure cultures. Complete records of only

9. Ztschr. f. Hyg. u. Infektionskrankh., 1902, 40, p. 73.

10. Ann. Rep. Med. Off. Loc. Govt. Bd., 1901-02, p. 404.

11. Ztschr. f. Hyg. u. Infektionskrankh., 1894, 11, p. 421.

12. Arch. f. Hyg., 1900, 37, p. 54.

13. Ztschr. f. Hyg. u. Infektionskrankh., 1905, 49, p. 135.

14. Sitzungsber. d. k. Akad. d. Wissensch., Math.-Naturwiss. Klasse, 1899, 108, p. 67.

15. München. med. Wchnschr., 1904, 51, p. 1283.

16. Arch. f. Hyg., 1905, 53, p. 128.

17. Centralbl. f. Bakteriöl., Orig., 1911, 60, p. 174.

18. Harvey Society Lectures, 1906-07, p. 64.

19. Centralbl. f. Bakteriöl., Ref., 1909, 44, p. 609.

20. Personal Communication sent to bacteriologists in the United States, 1912.

thirty organisms were kept, the others being lost. These thirty strains were obtained from the following sources: from human stools, 19 (normal stools of adults, 2; diarrheal stools of adults, 1; normal stools of infants, 5; diarrheal stools of infants, 4; stools of patients with pernicious anemia, 5; stools of patients with typhoid fever, 2); from the soil, 2; from sewage, 1; from milk, 1; from bird feces, 1; from cow feces, 3; from the lumen of a normal appendix obtained at autopsy, 1; from the washings from vegetables (potatoes and lettuce) bought in open market, 2.

The basis of the proposed classification is the fermentation reaction of the bacillus welchii in inulin and glycerin broths and its ability to produce spores in neutral media containing these substances. Because of the well-known ability of this organism to produce gas from sugar-free broth, the mere presence of gas cannot be taken as an indication of the ability of a given strain to attack inulin, glycerin, or other substances in such broth. In these experiments, it was considered that the organism had attacked the carbohydrate only when there was a definite increase of acidity along with the production of gas. In no instance was there more than 20 percent of gas when there was no increase in acidity. Even in the presence of a small amount of gas, if there was no increase in acidity of the medium and especially if spores were found to be present, it was believed that the strain in question had not acted upon the carbohydrate.

Upon the basis of their action upon glycerin and inulin broths, organisms belonging to the bacilli welchii group may be divided into four subgroups:

Subgroup 1.—Ferment both inulin and glycerin with production of gas and increase of acidity. Do not form spores in media containing either substance. Produce strong hemolysins and are pathogenic for guinea-pigs, even after many months' cultivation upon artificial media.

Subgroup 2.—Produce acid and gas from glycerin but not from inulin. Form spores in inulin, but not in glycerin broth. Hemolytic and pathogenic powers variable.

Subgroup 3.—Produce acid and gas from inulin but not from glycerin. Form spores in glycerin but not in inulin broth. Hemolysis and pathogenicity variable.

Subgroup 4.—Do not produce acid or gas from either inulin or glycerin, and form spores in both inulin and glycerin broths.

The source of culture gives no indication as to the subgroup to which it belongs, as shown by the grouping of the following twenty strains:

Subgroup 1.—Four strains. Normal stool, adult; diarrheal stool, adult; normal stool, three-day-old infant; diarrheal stool, infant.

Subgroup 2.—Seven strains. Soil, Brenham, Texas; normal stool, six-day-old infant; stool, adult with pernicious anemia; cow feces — three specimens; milk.

Subgroup 3.—Five strains. Normal stool, adult; stool, adult with pernicious anemia; stool, adult with typhoid fever; diarrheal stool, infant; normal appendix, autopsy.

Subgroup 4.—Four strains. Boston street dust; stools, patients with pernicious anemia — three specimens.

While the source of a culture does not determine the group to which it belongs, there may be some significance in certain facts shown in the tabulation: (1) All the strains isolated from cow feces and the one isolated from milk belonged to Subgroup 2. (2) Of the strains isolated from diarrheal stools, none belonged to Subgroup 2. The facts here presented are too few to justify a positive statement, but the possibility may be suggested that the variety of *Bacillus welchii* found in the intestinal tract of cows and in milk is not capable of causing gas bacillus diarrhea. If this is true, the human gas bacillus carrier assumes a more serious importance in the spread of this infection. (3) The strains of the *bacillus welchii* from the stools of patient with pernicious anemia tend to fall into Subgroup 4.

SUMMARY

The fermentation reactions in and the ability or inability to form spores in glycerin and inulin broths appear to furnish a reasonable and dependable means of dividing bacteria of the *bacillus welchii* group into four subgroups.

The source of a culture is no indication of the subgroup to which it belongs. It may be significant that all of the strains isolated from cow feces belonged to a group which contained none of those isolated from diarrheal stools. In view of this, it is suggested that the human gas bacillus carrier may prove to be of greater importance in the spread of this infection than milk infected with this organism from bovine sources.

The majority of those strains isolated from the stools of patients with pernicious anemia belonged to the same subgroup.

THE EFFECT OF SYMBIOSIS UPON SPORE FORMATION BY *BACILLUS WELCHII*, WITH SPECIAL REFER- ENCE TO THE PRESENCE OF THESE SPORES IN STOOLS *

J. P. SIMONDS

(From the Department of Bacteriology of the University of Texas, Galveston, and the Department of Pathology of Northwestern University Medical School, Chicago)

The surgical importance of the bacillus welchii has been recognized since Fraenkel¹ showed that it was the cause of gas phlegmon. Its significance for the internist became evident when it was shown by Tissier,² Passini,³ and, more conclusively, by Kendall and his coworkers⁴ that this organism may be the cause of severe diarrhea especially in infants, but also in adults.

The usual method of detecting the bacillus welchii in the stools is by means of the heated milk test, first used by Botkin,⁵ and perfected and employed very extensively by Kendall.⁶

The stools of infants suffering from gas bacillus diarrhea may be acid in reaction and yet contain large numbers of spores of this organism. The addition of sugar to the diet of such infants causes a serious and marked aggravation of the symptoms and an increase in the number of spores in the stools. Since it is well known that in artificial media the bacillus welchii does not sporulate except when such media are neutral or slightly alkaline and free from sugar, it seemed advisable to determine whether or not this bacillus could form spores in the presence of free acid or fermentable sugar in symbiosis with other organisms. Sittler⁷ has stated that the gas bacillus can produce spores in symbiosis with the colon bacillus under conditions and in media in which it would otherwise be unable to sporulate.

A number of different strains of the bacillus welchii, isolated from a variety of sources, were grown in broth and in sterilized suspensions of feces, with and without the addition of dextrose, in symbiosis with

* Received for publication September 22, 1914.

1. Ueber Gasphlegmone, Hamburg, 1893.

2. Ann. de l'Inst. Pasteur, 1905, 19, p. 273.

3. Ztschr. f. Hyg. u. Infektionskrankh., 1905, 49, p. 135.

4. Boston Med. and Surg. Jour., 1910, 163, p. 578; Ibid., 1912, 166, p. 75; Ibid., 1913, 169, p. 741.

5. Ztschr. f. Hyg. u. Infektionskrankh., 1894, 11, p. 421.

6. Ibid.

7. Centralbl. f. Bakteriolog. Orig., 1908, 47, pp. 14, 145.

B. coli, *B. subtilis*, and *B. prodigiosus*. In no instance, did the symbiosis have any effect upon the spore-forming powers of any of the strains used.

The influence of the mixed fecal flora was next determined. The stools of a normal, healthy adult, which contained from 10-2,000 spores of the bacillus welchii per gram of dried feces, were available for systematic study. At frequent intervals, suspensions were made of these stools in sterile salt solution. The suspension was distributed in

TABLE 1

SHOWING THE INFLUENCE OF THE MIXED FECAL FLORA UPON SPORULATION BY THE *BACILLUS WELCHII* IN THE PRESENCE OF FERMENTABLE SUGARS

Fecal Suspension	Hours* Incuba- tion	Acidity* (Phenolph- thaleïn)	Dilutions†									
			1-100	1-200	1-400	1-800	1-1,600	1-3,200	1-6,400	1-12,800	1-25,600	1-51,200
Fresh.....	+	+	+	+	+	-	-	-	-	-
Plain.....	24	0.4	+	+	+	+	+	+	+	+	+	+
With 1 percent lactose..	24	3.0	+	+	+	+	+	+	+	+	+	-
Fresh.....	+	+	+	-	-	-	-	-	-	-
Plain.....	24	0.6	+	+	+	+	+	+	+	+	-	-
With 1 percent dextrose	24	5.4	+	+	+	-	-	-	-	-	-	-
With 1 percent lactose..	24	5.4	+	+	-	+	-	-	-	-	-	-
With 1 percent maltose.	24	4.4	+	+	+	-	-	-	-	-	-	-
Fresh.....	+	+	-	-	-	-	-	-	-	-
Plain.....	48	0.4	+	+	+	+	+	+	+	+	+	+
With 1 percent lactose..	48	2.6	+	+	+	+	+	+	+	+	+	+
With 1 percent dextrose	48	2.6	+	+	+	+	+	+	+	+	+	+
With 1 percent maltose.	48	2.6	+	+	+	+	+	+	+	+	+	+

* Expressed as the number of cubic centimeters of N/10 NaOH required to neutralize 100 c.c. of suspension.

† The sign + means that stormy fermentation was present, and that there was at least one spore in 1 c.c. of the dilution used.

50 c.c. amounts in large test tubes. In each lot, one tube of suspension was left without further treatment. To the others, there was added enough sterile solution of dextrose, lactose, or maltose, to make a 1 percent solution. The highest dilution of the suspension, which would produce stormy fermentation of milk when 1 c.c. was inoculated into a tube of milk and heated to 80 C. for fifteen minutes, was determined.

The tubes of suspension were then placed in a large desiccator or Novy jar in the bottom of which was a quantity of pyrogallie acid wrapped in filter paper. Fifty to one hundred cubic centimeters of strong potassium hydroxid solution were then poured through a long

funnel into the jar which was then quickly closed. The air was strongly exhausted by means of a vacuum pump.

After twenty-four to forty-eight hours' incubation, the contents of each tube were thoroughly mixed and the highest dilutions, 1 c.c. of which would cause stormy fermentation of milk after heating to 80 C. for fifteen minutes, were determined for each tube. The occurrence of stormy fermentation in any tube of milk was considered proof of the presence of one or more spores of the *bacillus welchii* in 1 c.c. of the dilution of the suspension used.

The results of three characteristic experiments are shown in Table 1.

SUMMARY

In a plain suspension of feces containing the *bacillus welchii*, kept at 37 C. under anaerobic conditions for twenty-four to forty-eight hours, there is a marked increase in the number of spores of this organism.

In the presence of the mixed fecal flora, the *bacillus welchii* may sporulate even in the presence of free acid or fermentable carbohydrate.

The character of the associated flora is the most important factor determining the power of sporulation. When acid formers are so abundant that the acidity becomes such that 3 c.c. or more of N/10 NaOH are required to render 100 c.c. of medium neutral to phenolphthalein, there is no increase in the number of spores. When, on the other hand, acid-producing bacteria are absent or few in number so that less than 3 c.c. of N/10 NaOH will render 100 c.c. of suspension neutral, the *bacillus welchii* is able to produce spores in abundance.

That this ability to sporulate in the presence of free acid or fermentable sugar depends upon symbiotic relations of the *bacillus welchii* with one or more species, other than the colon bacillus, is indicated by the negative results obtained when a pure culture of the *bacillus welchii* was grown in symbiosis with a pure culture of the colon bacillus.

THE ACTION OF SODIUM SULPHOCYANATE IN
TUBERCULOSIS
STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF
TUBERCULOSIS, XII*

HARRY J. CORPER

*(From the Otto S. A. Sprague Memorial Institute and the Pathological Laboratory of the
University of Chicago.)*

A chemotherapeutic agent, to be of value in tuberculosis, must either produce a "Therapia magna sterilisans" in the Ehrlich sense, or must inhibit the development of the tubercle bacillus to such an extent that further growth is impossible. In order to produce either of the above, it is necessary for the chemotherapeutic agent to enter the diseased tissues in concentrations sufficient to produce either a bactericidal or an inhibitory effect, and this without material injury to the host. It is conceivable that a chemotherapeutic agent, which enters the diseased tissues, may enter, as do crystalloids, in concentration nearly equal to that in the blood, or may enter in low concentrations harmless to the host and gradually accumulate in the diseased tissues in concentrations to be bactericidal or inhibitory, altho the latter is not highly probable since a chemotherapeutic agent, which enters a tissue by way of the blood, will probably also pass out the same way as the concentration in the blood diminishes. Since we are aware, however, of such conditions as chemical affinities, this factor must be borne in mind as a possible reality. It is known, at least, that calcium salts are deposited in old tubercles and remain there in concentration far above that in the blood or body fluids. If the chemotherapeutic agent enters in concentration nearly equal to that in the blood, it should remain there, without harm to the host, in concentration sufficient to be bactericidal for a sufficient length of time, or if in concentration sufficient to be inhibitory, repeated introduction must keep up the inhibitory concentration until a permanent effect has been produced or until the host is in such a condition that further immediate development of the disease is impossible.

With these facts in mind, the study of a number of chemotherapeutic agents was undertaken, among these sodium sulphocyanate.

* Received for publication September 23, 1914.

Sodium sulphocyanate was chosen on account of its almost complete lack of toxicity toward animals, its relation to the highly toxic cyanids, its simple crystalline character, and its simplicity in chemical determinations.

LITERATURE

The toxicity of the alkali sulphocyanates was studied thoroughly by Franz,¹ who believes that they possess no toxic action toward the animals studied (guinea-pig, rabbit, dog, and cat) not attributable to mere salt action, and concludes that they cannot be considered poisons in the ordinary sense. The lethal dose of potassium sulphocyanate to rabbits, given per os, was about 0.5 gm. per kilo, which was fatal in 3.5-4 days (0.9 gm. was fatal in about 6 hours). Guinea-pigs, given per os 0.6 gm. sodium sulphocyanate per kilo, died in 0.5-1 day, while 0.8 gm. was fatal in about 4 hours. One gram potassium sulphocyanate, given subcutaneously to dogs (weighing 5.7-7.8 kilos), caused no toxic symptoms. The intravenous injection of 0.25-0.5 gm. sodium sulphocyanate into dogs (6-8 kilos) was without effect.

The fact that potassium sulphocyanate was distributed, after feeding, throughout nearly the entire organism was noted by Albert,² tho only in a qualitative way. He states: "I have tried the ingestion of pure potassium sulphocyanid (sulphocyanate) in four rabbits, using daily between 1 and 2 gm. mixed with about ten of bran. In every case, death resulted in about a week. The symptoms produced were alike, viz., emaciation and loss of hair. In two, a marked atrophy of the salivary glands was found, and in one of these, after dipping in a solution of ferric perchlorid, a microscopic examination was made, the ducts being found more deeply stained than the gland tissue. In all four, large quantities were found in the urine and feces; in fact, the drug seemed to permeate, as far as one was able to judge, every organ and tissue, and to find its way in every secretion and excretion even to the cerebrospinal fluid."

Pollak³ noted that, after the subcutaneous administration of sodium sulphocyanate to dogs (about 1 gm.) and rabbits (0.2-0.5 gm.), and when given per os to dogs (about 1 gm.) and man (about 2 gm.), the entire quantity was excreted in the urine in from 4-5 days. The animals tolerated the above amounts without reaction. DeSouza⁴ observed that sulphocyanates pass from the blood into the saliva, pancreatic juice, bile, and urine. The concentration in the urine may be greater or less than that in the blood. After feeding sodium sulphocyanate, 82, 26.7, and 39.8 percent of that given with the food was still in the body 21, 44 and 47 hours, respectively, after ingestion. In one experiment on a dog, 22.5 percent of 3 gm. given with food remained in the body 72 hours after ingestion. Edinger and Clemens⁵ analyzed the organs of a man, given 0.5 gm. sodium sulphocyanate daily for 5 days before death, and found 2 mg. in the liver, 14 mg. in the pancreas, 11 mg. in the kidneys, and practically none in the salivary glands.

Two conflicting reports were found in the literature on the effect of sodium sulphocyanate on tuberculosis; one by Martinotti,⁶ who states that tubercle bacilli (variety not given) were injected into the anterior chambers of rabbits' and guinea-pigs' eyes, typical tubercles were allowed to develop and the animals

1. Arb. a. d. k. Gsndhtsamte., 1911, 38, p. 435.

2. Lancet, 1898, 1, p. 494.

3. Beitr. z. chem. Phys. u. Path., 1902, 2, p. 430.

4. Jour. Physiol., 1906, 35, p. 332.

5. Ztschr. f. klin. Med., 1906, 59, p. 218.

6. Centrallbl. f. Bakteriöl., Abt. I, 1896, 19, p. 142.

were then treated with sodium sulphocyanate, subcutaneous injections two or three times daily (amounts not given). The animals were completely cured and when after many months they were killed, they revealed no trace of tuberculous infection either in the eye or other organs, while controls rapidly died. The other report was by Treupel and Edinger⁷ whose associate, Schlegel, found that, in culture experiments on serum and glycerin agar, tubercle bacilli (variety not given) grew vigorously in the presence of 0.6, 0.12, and 0.25 percent sodium sulphocyanate, but in the presence of 0.5 and 1 percent only sparse growth occurred. In animal experiments on guinea-pigs and rabbits, entirely negative results were obtained. The guinea-pigs were given subcutaneous injections of 0.01 gm. sodium sulphocyanate daily for five to six weeks before and continued after infection in one series, and five injections of 0.025 gm. daily after infection in another series, while the rabbits received subcutaneously 0.1 gm. sodium sulphocyanate daily for five weeks previous to and continued after infection. The type of tubercle bacilli was not stated, but was probably the bovine bacilli as indicated by the character of the disease produced in the animals.

TOXICITY OF SODIUM SULPHOCYANATE

On account of the lack of accurate data in the literature (at least none such was found) on the intravenous toxicity of sodium sulphocyanate, it was necessary to determine this in order to obtain our

TABLE 1
INTRAVENOUS TOXICITY OF SODIUM SULPHOCYANATE TO RABBITS*

Weight of Animal in Grams	Amount of Injection	Amount in Grams per Kilo	Results
1,300.....	0.3 gm. in 3.0 c.c.	0.23	Alive on fourth day, emaciated (950 gm.). Dead on fifth day
2,100.....	0.75 gm. in 7.5 c.c.	0.35	Lost weight (fourth day 1,750 gm.)
(17 days later)	1.00 gm. in 10 c.c.	0.47	(1,900 gm.). Alive
1,350.....	0.5 gm. in 5 c.c.	0.37	Much emaciated, dead on eighth day
1,300.....	0.5 gm. in 5 c.c.	0.38	(Fourth day 1,300 gm.). Dead fifth day after last injection
(11 days later)	1.0 gm. in 10 c.c.	0.76	
2,400.....	1.0 gm. in 10 c.c.	0.41	Alive.
2,240.....	1.0 gm. in 10 c.c.	0.44	Dead in 14 hours and 45 minutes
2,000.....	1.0 gm. in 10 c.c.	0.50	Dead on fourth day
1,740.....	1.0 gm. in 10 c.c.	0.57	Dead in 8 hours and 45 minutes
1,700.....	1.0 gm. in 10 c.c.	0.58	Alive
1,700.....	1.0 gm. in 10 c.c.	0.58	Alive
1,250.....	0.75 gm. in 7.5 c.c.	0.60	(Fourth day 1,200 gm.). Alive
(11 days later)	1.0 gm. in 10 c.c.	0.80	
2,270.....	1.5 gm. in 15 c.c.	0.66	Dead in 4 hours and 25 minutes
1,300.....	1.0 gm. in 10 c.c.	0.76	Dead in less than 16 hours
1,740.....	1.5 gm. in 15 c.c.	0.86	Dead in 1 hour and 45 minutes
2,240.....	2.0 gm. in 20 c.c.	0.89	Dead in 1 hour and 20 minutes
1,890.....	2.0 gm. in 20 c.c.	1.05	Dead in 2 minutes

* In all the experiments in this paper, it was considered sufficiently accurate to use pure crystals of sodium sulphocyanate (which show no signs of deliquescence and have been kept in paraffin-stoppered bottles), and all the weights given indicate the amount of pure crystals rather than the absolute amount of sulphocyanate which could be obtained only by frequent quantitative chemical analyses. Therefore, the figures are relative rather than absolute.

figures on the highest concentration that can be attained in the tissues without material injury to the host. With a simple crystalline salt, as sodium sulphocyanate, the intravenous lethal dose is the only gauge that can be used, since it is desirable to avoid the local toxic action such as might occur by giving large amounts per os or subcutaneously. By the intravenous route, an almost immediate dilution of the salt occurs and thus only the systemic toxicity is obtained. The results of these experiments are shown in Table 1.

The principal immediate symptoms noted, when toxic doses of sodium sulphocyanate were given, were a muscular rigidity and paralysis of the hind legs, irregular, rapid, or slow-labored respiration, and toward the end, when death occurred, coma. Emaciation was one of the principal remote symptoms when the animal survived for a few days.

Table 1 shows that the acute lethal dose for a 1 kilo rabbit was about 0.4-0.6 gm. of sodium sulphocyanate, given intravenously. Delayed death (after the fifth day) may occur in exceptional cases, however, even with smaller doses.

CONCENTRATION OF SODIUM SULPHOCYANATE IN THE TISSUES

With the systemic lethal dose of sodium sulphocyanate now known, it was possible to determine the maximum concentration obtainable in the tissues, mainly the tubercles.

Method of Analysis.—The following quantitative colorimetric method of analysis was used because of its simplicity and sufficient relative, rather than absolute, accuracy to determine the large amounts of sodium sulphocyanate which interest us in this problem (absolute accuracy not being necessary).

The sample of tissue (1-5 gm.) to be analyzed was placed in about 75-100 c.c. of 95 percent alcohol and ground up, allowed to remain in a cool place with frequent shaking for twenty-four hours, and then filtered through a dry filter paper, the precipitate washed two or three times with small amounts of 95 percent alcohol, the filtrate evaporated to dryness on the water bath and, after cooling, the residue extracted (by stirring with a glass rod) by means of 3-4 portions of 3-5 c.c. of 95 percent alcohol, and filtered. To the filtrate (or fraction thereof) diluted to a definite volume (10 c.c.) was added 0.1 c.c. ferric chlorid (30 percent) solution and it was vigorously shaken. The addition of the ferric chlorid frequently results in the formation of a turbidity, which separates on shaking or may be removed by filtration through a dry filter paper. The resulting solution was then placed in the chamber of a DuBosc colorimeter and compared with a standard solution of sodium sulphocyanate in 95 percent alcohol, containing from 0.2-2.0 mg. sodium sulphocyanate in 10 c.c., to which had been added 0.1 c.c. ferric chlorid solution (30 percent).

The delicacy of this method decreased with decreasing amounts of sodium sulphocyanate on account of the interfering color produced by the 0.1 c.c. (30 percent) ferric chlorid. Tested in the DuBosc colorimeter, using 10 c.c. 95

percent alcohol as solvent, this color corresponded to a reading of 0.04 mg., using 0.2 mg. sodium sulphocyanate for comparison. The interference was barely appreciable when over 0.2 mg. sodium sulphocyanate was present.

Control Analysis to Test the Accuracy of the Method.—In order to test the accuracy of the method just described, 1.0 mg. sodium sulphocyanate was added to samples of a rabbit's organs and these were then analyzed. The results were as follows:

6.5 and 7.5 gm. of blood yielded, respectively....	1.0 and 0.95 mg. NaSCN.
5.0 and 5.0 gm. of liver yielded, respectively....	0.77 and 0.60 mg. NaSCN.
6.0 and 6.5 gm. of kidney yielded, respectively....	0.86 and 0.85 mg. NaSCN.
5.2 and 3.7 gm. of lung yielded, respectively....	0.84 and 0.76 mg. NaSCN.
2.8 and 2.8 gm. of eye yielded, respectively....	0.80 and 0.91 mg. NaSCN.
5.2 gm. of heart yielded.....	0.80 mg. NaSCN.

Controls, using 4.5 gm. of tissue (kidney, heart, and lung) to which had been added 0.4 and 0.6 mg. sodium sulphocyanate, carried through the entire method, revealed a recovery of about 70 percent.

Duration of Sodium Sulphocyanate in the Blood After Intravenous Injection.—A rabbit (2,100 gm.) was given in the marginal ear vein 0.75 gm. of sodium sulphocyanate in 7.5 c.c. of distilled water fifteen minutes after withdrawing 5 gm. of blood from the heart for control analysis. At intervals thereafter, other samples were taken from the heart and analyzed for sodium sulphocyanate content colorimetrically by the method given above with the following results:

The normal sample contained no sulphocyanate.

4.1 gm. taken thirty minutes after injection of the sodium sulphocyanate contained 0.88 mg. per gram of blood.

4.6 gm. taken eighteen hours after injection contained 0.59 mg. per gram.

4.3 gm. taken twenty-three hours after injection contained 0.57 mg. per gram.

7.1 gm. taken 66 hours after injection contained 0.47 mg. per gram.

6.7 gm. taken 92 hours after injection contained 0.23 mg. per gram.

7.1 gm. taken 116 hours after injection contained 0.014 mg. per gram.

7.0 gm. taken 140 hours after injection contained 0.000 mg. per gram.

This experiment shows that sodium sulphocyanate (0.36 gm. per kilo) given intravenously to rabbits is present in the blood in appreciable amounts up to the fifth day after administration.

As sodium sulphocyanate given intravenously can be present in the blood for at least 5 days, it is significant in what concentrations and how long it remains in the tissues of the body. Is there any evidence of a chemical affinity for any of these tissues? With these questions in mind the following experiments were performed.

The analyses were carried out in duplicate when enough tissue was available for this purpose (that is, provided the organs analyzed weighed more than 3.5 gm.).

Experiment 1.—A rabbit (2,500 gm.) with a well-developed tuberculosis* of the right eye involving the entire bulb was given intravenously, into the

* In all the experiments in this paper the human type of tubercle bacilli was used.

marginal ear vein, 1 gm. of sodium sulphocyanate in 10 c.c. sterile distilled water and was bled to death four hours after the injection. All the organs were normal. The tissues were analyzed with the following results: The blood contained 0.82 mg. sodium sulphocyanate per 1 gm.; the liver (66 gm.) 0.20 mg.; the left lung (3.0 gm.) 0.52 mg.; the right lung (4 gm.) 0.50 mg.; the heart (7 gm.) 0.46 mg.; right kidney (8 gm.) 0.30 mg.; left kidney (8 gm.) 0.30 mg.; testes (6 gm.) 0.46 mg.; tuberculous right eye (4 gm.) 0.67 mg.; and the normal left eye (4 gm.) 0.45 mg.

Experiment 2.—A rabbit (2,420 gm.) was injected intramuscularly with dead fat-free tubercle bacilli (which produced a typical sterile tubercle) and, after development of nodules of 4-5 gm., was given intravenously 1 gm. sodium sulphocyanate in 10 c.c. sterile distilled water. The animal was bled to death twenty-four hours after the injection of the sodium sulphocyanate and the tissues were analyzed with the following results: The blood taken fifteen minutes before injection (for control) contained no sulphocyanate, one hour after injection 0.90 mg. per 1 gm., twenty-four hours (when the animal was killed) after injection 0.62 mg.; the liver (52 gm.) contained 0.25 mg.; right lung (4.7 gm.) 0.46 mg.; left lung (3.5 gm.) 0.57 mg.; heart (5.5 gm.) 0.40 mg.; right kidney (6.8 gm.) 0.35 mg.; left kidney (7.0 gm.) 0.27 mg.; the urine found in the bladder 0.60 mg.; testes (7.4 gm.) 0.36 mg.; normal right eye (3.3 gm.) 0.51 mg.; normal left eye (3.0 gm.) 0.52 mg.; a necrotic nodule in the left fore leg (4.4 gm.) 0.75 mg.; a necrotic nodule in the right fore leg (4.0 gm.) 0.65 mg.; normal muscle from the left fore leg 0.08 mg.; normal muscle from the right fore leg 0.08 mg.; and normal muscle from left hind leg 0.10 mg.

Experiment 3.—A rabbit (2,800 gm.) with a fairly well developed tuberculosis of the right eye was given intravenously 0.9 gm. sodium sulphocyanate in 9 c.c. sterile distilled water and was bled to death three days after the injection. All the organs were normal. The tissues were analyzed with the following results: The blood contained 0.65 mg. sodium sulphocyanate per 1 gm.; liver (52 gm.) 0.28 mg.; right lung (3.8 gm.) 0.52 mg.; left lung (3.5 gm.) 0.47 mg.; heart (4.8 gm.) 0.37 mg.; right kidney (5.2 gm.) 0.32 mg.; left kidney (5.5 gm.) 0.37 mg.; the tuberculous right eye (3.2 gm.) 0.56 mg.; normal left eye (3.4 gm.) 0.53 mg.; normal muscle of right hind leg 0.06 mg.; and the normal muscle of the left hind leg 0.06 mg.

Experiment 4.—A rabbit (2,830 gm.) with a well-developed tuberculosis of the right eye was given intravenously 0.9 gm. sodium sulphocyanate in 9 c.c. sterile distilled water and died four days later. The left lung contained 0.38 mg. sodium sulphocyanate per 1 gm.; the right lung 0.38 mg.; right kidney 0.26 mg.; left kidney 0.24 mg.; the tuberculous right eye 0.46 mg.; and the normal left eye 0.41 mg.

Experiment 5.—A rabbit (2,800 gm.) was injected intramuscularly with dead fat-free tubercle bacilli, and, after development of good-sized nodules, was given intravenously 0.75 gm. sodium sulphocyanate in 7.5 c.c. sterile distilled water. The animal was bled to death five days after the injection of the sodium sulphocyanate and the tissues were analyzed with the following results: The blood contained 0.03 mg. sodium sulphocyanate per 1 gm.; the liver (89 gm.) 0.01 mg.; right lung (6.5 gm.) 0.02 mg.; left lung (5.6 gm.) 0.02 mg.; heart (7.5 gm.) 0.02 mg.; mammary glands 0.02 mg.; right kidney (8.4 gm.) 0.02 mg.; left kidney (8.6 gm.) 0.03 mg.; normal right eye (3.5 gm.) 0.03 mg.; normal left eye (4.0 gm.) 0.03 mg.; a necrotic nodule in the right fore leg (9.7 gm.) 0.02 mg.; a necrotic nodule in the left fore leg (13.5 gm.) 0.02 mg.; a necrotic

nodule in the right hind leg (7.5 gm.) 0.02 mg.; caseous material (4.0 gm.) from a nodule in the left hind leg 0.04 mg.; capsule (3.5 gm.) of this nodule 0.04 mg.; and normal muscle from the right hind leg 0.01 mg.

Experiment 6.—A rabbit (2,400 gm.) with a large acute abscess was given 1.0 gm. sodium sulphocyanate intravenously and bled to death after two days. the blood contained 0.57 mg. sodium sulphocyanate per 1 gm. and the pus contained 0.59 mg.

Experiments 7 and 8.—Two guinea-pigs (about 400 gm.) with well-advanced generalized tuberculosis (enlarged inguinal, retroperitoneal, and peribronchial lymph glands, liver full of numerous necrotic areas, spleen enlarged and containing large areas of necrosis, and lungs containing numerous small foci of necrosis) were given subcutaneously 0.5 gm. sodium sulphocyanate in 5 c.c. sterile distilled water and killed about three hours after the injection of the

TABLE 2

DISTRIBUTION OF SODIUM SULPHOCYANATE IN THE TISSUES OF TUBERCULOUS RABBITS AT VARIOUS INTERVALS AFTER INTRAVENOUS INJECTIONS

Experi- ment	NaSCN Given per Kilo	Time Between Anal- ysis and Injec- tion	Amount of Sulphocyanate in Milligrams												
			Blood	Liver	Right Kid- ney	Left Kid- ney	Heart	Testes	Right Lung	Left Lung	Normal Eye	Tuber- cular Eye	Normal Muscle	Tuber- culous Nodule from Muscle	Pus
1.....	0.4	4 hrs.	0.82	0.20	0.30	0.30	0.46	0.46	0.50	0.52	0.45	0.67
2.....	0.41	24 hrs.	0.62	0.25	0.35	0.27	0.40	0.36	0.46	0.57	R. 0.51 L. 0.52	0.08 0.08 0.10	0.75 0.65
3.....	0.32	3 days	0.65	0.28	0.32	0.37	0.37	0.52	0.47	0.53	0.56	0.06 0.06
4.....	0.32	4 days	0.26	0.24	0.38	0.41	0.46
5.....	0.27	5 days	0.03	0.01	0.02	0.03	0.02	0.02	0.02	R. 0.03 L. 0.03	0.01	0.02 0.02 0.04
5.....	0.41	2 days	0.57	0.59

sodium sulphocyanate. The tissues were analyzed with the following results: The blood contained 1.66 and 1.04 mg. sodium sulphocyanate per 1 gm.; the liver (16 and 25 gm.) 0.62 and 1.11 mg.; the spleen (3 and 2.5 gm.) 0.52 and 0.80 mg.; lungs (4 and 8 gm.) 0.62 and 0.62 mg.; kidneys (3.0 and 3.5 gm.) 0.50 and 0.46 mg.; the inguinal glands (2.5 and 2.5 gm.) 0.52 and 0.64 mg.; the retroperitoneal glands (1.0 and 1.5 gm.) 1.56 and 1.40 mg.; and the peribronchial glands (1.5 and 1.5 gm.) 1.0 and 1.1 mg., respectively.

Sodium sulphocyanate, a simple crystalloid, given intravenously to rabbits is therefore found in high concentration, about the same as that of the blood, in the tubercle (tuberculous eye of the rabbit, necrotic tissues produced by dead fat-free tubercle bacilli in the muscles of rabbits, and tuberculous tissues of the guinea-pig), the normal eye,

lungs, kidneys, heart, and testes. The liver generally contains less than the blood and other organs, and the normal muscle contains only traces. These results agree well with those of Wells and Hedenburg,⁸ who found that simple crystalloids (potassium iodid) enter necrotic tissues to reach about the same concentration as in the blood. They also bear evidence in favor of the statement made by these authors that simple crystalloids are well suited as entrants into the tubercle. Pus from acute abscesses in rabbits also contains large amounts of sodium sulphocyanate, about equal to the amount in the blood after injection. The sodium sulphocyanate, in the tissues and tubercles, disappears as rapidly as it does from the blood and is practically absent after 5 days. No evidence of a chemical affinity for any of the tissues was obtained.

TUBERCULOCIDAL ACTION OF SODIUM SULPHOCYANATE

In order to determine whether or not sodium sulphocyanate possessed any bactericidal properties, 10 drops of a heavy emulsion of tubercle bacilli were added to 5 c.c. of varying concentrations—1.0, 0.5, 0.1, 0.01, and 0.001 percent of sodium sulphocyanate (distilled water was used as solvent in the higher and 0.9 percent salt solution in the lower concentrations) in duplicate and placed in the incubator at 37 C. for 48 hours. Six controls were made at the same time, three with distilled water, and three with 0.9 percent sodium chlorid. At the end of this time, the entire solution (in the case of the higher concentrations, the sediment only was used) was injected subcutaneously into normal guinea-pigs. It suffices to state that all the guinea-pigs developed a tuberculosis. Even the 1 percent sodium sulphocyanate did not attenuate the bacilli in this length of time.

Similarly, human tubercle bacilli were exposed to 0.1, 0.01, and 0.001 percent sodium sulphocyanate for 7 days at 37 C., being injected into normal guinea-pigs at the end of this time. All of the guinea-pigs developed a tuberculosis.

SUMMARY

Sodium sulphocyanate is lethal to rabbits when given intravenously in amounts of 0.4-0.6 gm. per kilo. Delayed death may occur even from smaller amounts.

When injected intravenously (about 0.4 gm. per kilo), it is found in the tuberculous tissues in concentration about equal to that in the

8. Jour. Infect. Dis., 1912, II, p. 349.

blood (0.06-0.08 percent). The concentration in the lungs, heart, kidneys, and testes is not far from that in the blood, the concentration in the liver is less, while it is practically absent from the muscles. It disappears from the tissues (normal and tuberculous) as speedily as it does from the blood (being absent about 5 days after injection). No evidence of a chemical affinity of the sodium sulphocyanate for any of the normal or tuberculous tissues was obtained. Tubercle bacilli, exposed to concentrations of sodium sulphocyanate up to 1 percent for 48 hours at 37 C. and up to 0.1 percent for 7 days at 37 C., were not killed. No evidence even of attenuation was observed.

SODIUM TELLURITE AS A RAPID TEST FOR THE VIABILITY OF TUBERCLE BACILLI

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS, XIII *

HARRY J. CORPER

*(From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the
University of Chicago)*

A reliable agent which would indicate in a short period of time life or death of the tubercle bacillus and eliminate the animal test would be a valuable help in work on tuberculosis, especially on its experimental chemotherapy. It was hoped that such an agent was at hand when Gosio described his selenite and tellurite reduction tests as indicators of life or death of bacteria. In his tests, he included the tubercle bacillus and found that it behaved exactly as other bacteria towards the agent. If Gosio's statement is correct, one could accomplish within twenty-four to forty-eight hours what would require at least a month by the inoculation method. Since Gosio's tests on the tubercle bacillus, however, were only incidental in a large series, it was necessary in determining their practical value to control his results and to apply them to actual experiments in chemotherapy. If the tests could not be used in chemotherapeutic experiments where interfering chemical substances are dealt with, they perhaps could be an aid in the determination of whether a culture to be used in an experiment was viable or not. Absence of this information frequently occasions the loss of valuable time because where the culture is not viable, this becomes apparent only at the end of a month or so when neither controls nor test animals develop tuberculosis, or when, in culture experiments, no growth occurs. Further investigation was also indicated by the fact that the agents in question possess possible chemotherapeutic value.

LITERATURE

As a result of his work with the alkali selenites and tellurites, Gosio¹ concludes that these salts are valuable reagents for determining bacterial life as they are reduced by living bacteria, the reduction products staining the bacteria cells. The tellurites show a black stain, the selenites a red. He preferred

* Received for publication October 10, 1914.

1. Ztschr. f. Hyg. u. Infektionskh., 1905, 51, p. 65.

the tellurites as they are more permanent and because they are not so easily mistaken for interfering colors. To obtain the best action, it is essential that the microorganisms are growing well; when spores are forming or development is at a standstill, the test is uncertain and unreliable. The delicacy of the reaction is in direct relation to the amount of chemical reagent and the quantity of bacteria that can live in its presence. Therefore, it is essential that the chemical should not reach a dose toxic to the bacteria. All factors that increase the activity of the bacteria increase the reaction with the indicator, and vice versa. Not all media are equally favorable for the reaction; broth and milk proved best, serum inhibited the reaction, while a small amount of sugar increased it. Dead bacilli do not appreciably decompose the tellurite, for instance, with typhoid bacilli. With some bacteria, long contact and higher temperature often produce an ash-gray color. The salt acts plainly in dilutions of 1:100,000 and even 1:200,000, and retains its chemical properties for months in ordinary substrata in which it may be used. Aseptic reduction can occur, but only under extraordinary conditions (in the presence of reducing agents, high heat, vacuum, etc.). Injected into tissues of living animals, methyl telluride with its characteristic odor is formed, and all the nuclei and part of the cytoplasm are stained black with tellurium. Gosio examined one hundred and seventy-three microorganisms and divided them into three classes dependent upon the reaction obtained; (1) a decided reaction, (2) a less intense, but fully evident, reaction [in this class he placed a bovine, a human, and an avian tubercle bacillus and a so-called pseudotuberculosis bacillus (Rabinowitsch)], (3) very slight reaction.

Belfanti² studied the behavior of the reaction of Gosio with tubercle bacilli by adding to agar plates small amounts of tellurite (1:25,000-1:50,000) and found that lumps of culture of human, bovine and avian bacilli reduced the potassium tellurite markedly and, in a few hours, the bacilli were stained black. The reaction occurred within wide ranges of temperature; it was most marked at 37 C., was slightly less at room temperature, and developed slowly on ice. High concentrations (1 percent and 0.1 percent) of the tellurium salt destroyed the organisms, so that when transferred to suitable nutrient media they did not grow, or produced only a slight growth. Cultures exposed for a few minutes to ether or acetone vapors did not give the reaction. Belfanti suggested that these salts are bacteriostatic in the Ehrlich sense, and can be used for therapeutic study.

If the tellurite test for viability of tubercle bacilli is to be used in chemotherapeutic work, it must indicate the life or death of the bacilli after they have been treated with the chemical which is being tested for its tuberculocidal action. Under ideal conditions, it should be possible to add the tellurite directly to the chemical solution and obtain the desired test. Practically, however, this is impossible, as the tellurite is rather active chemically and is susceptible to decomposition or precipitation by a large number of substances. If the test could be carried out satisfactorily in distilled water or in 0.9 percent salt solu-

2. *Rev. Ist. Lomb. di sci. e lett. rendic.*, Milano, 1912, 45, p. 539; *Ztschr. f. Chemoth.*, Orig., 1912, p. 113.

tion, this objection would not be as great, but, as was pointed out by Gosio and will be shown in this paper, the reduction test is dependent, not so much upon life or death of the organism, as it is upon the fact that the organism is in active metabolism and present in sufficient number. Therefore, to obtain the test the organism must be suspended in a suitable nutrient medium. Another course is open and that is to suspend the tubercle bacilli in the solution to be tested for its tuberculocidal action, wash the bacilli free from this solution with sterile salt solution or distilled water, add the tellurite in sterile broth to the washed bacilli, and then incubate. This would prove ideal if it were not for the fact that the washing process and the centrifugation can rarely be carried out without contamination by rapidly growing organisms which also reduce the tellurite and have the ability of rapidly overgrowing the tubercle bacilli. As will be shown later, tubercle bacilli which have been emulsified are very slow in reducing the tellurite or may even not do so unless they are present in very large amounts.

In spite of the fact that the tellurite test could not be used as a reliable index of life or death of the tubercle bacillus in tuberculocidal experiments, it nevertheless proved of value as a rapid index of life or death of cultures of tubercle bacilli if the tests were properly carried out. The following experiments led to a method which would reveal in from one to two hours whether a culture of tubercle bacillus was viable or not.

A series of tubes were made containing 5 c.c. of 0.9 percent salt solution and dilutions of sodium tellurite 1:1,000, 1:1,500, 1:7,500, 1:10,000, 1:15,000, 1:75,000, 1:100,000, 1:150,000, and 1:750,000. To each of these were added a few drops of a fairly heavy emulsion of tubercle bacilli in salt solution, containing 5-10 mg. bacilli to 10 c.c. of the solution, and placed in the incubator at 37 C. No visible reduction occurred in any of these within seventy-two hours. Similar dilutions were then made and to them were added, instead of an emulsion, lumps of tubercle bacillus cultures. They were placed at 37 C. No visible reduction occurred in any of the dilutions up to 1:100,000 in seventy-two hours; 1:150,000 gave a slight reduction in forty-eight hours, and this only in a lump of culture on the surface of the liquid, and 1:750,000 gave a good reduction, but not until after forty-eight hours.

Emulsified tubercle bacilli in glycerin broth, unless present in larger amounts with the same dilutions of tellurite used above, gave about similar results as with the salt solution. Lumps of culture in glycerin broth gave a reduction in dilutions from 1:100,000, etc., but not appearing until after about twenty-four hours at 37 C.

If the tubercle bacilli are present in sufficient amount, a reduction of the tellurite is obtained even in salt solution. To 3 c.c. of a heavy emulsion of

tubercle bacilli were added varying amounts, 0.01, 0.05, 0.1, and 0.2 mg. of sodium tellurite. This was incubated at 37 C. A reduction occurred after twenty-four hours in all except the one containing 0.01 mg.

A series of sterile, hollow, ground glass slides were prepared and in the bowl of each a small lump of culture of tubercle bacillus was placed. To each was added a drop of varying concentrations (1:500, 1:5,000, 1:50,000, etc.) of sterile sodium tellurite in distilled water and covered by means of a sterile cover glass bordered with sterile vaselin to prevent drying of the culture. The slides were then placed in the incubator at 37 C. The one to which had been added one drop of 1:500 sodium tellurite gave a reduction within thirty minutes to one hour, being completely black in a few hours. The 1:5,000 sodium tellurite test gave only a faint reduction as compared to the 1:500, not appearing before twelve to twenty-four hours, and the 1:50,000 did not give a visible reduction even in forty-eight to seventy-two hours.

Two questions immediately arose as a result of these experiments: Whether or not emulsified bacteria could, by some means, be concentrated and the concentrated residue be used for this rapid test, for tuberculocidal work (contaminators would not develop rapidly enough to interfere with a test occurring in so short a time): and whether or not this test was really an index of viability of the organism.

The former question was answered as follows: A heavy emulsion of tubercle bacilli was made and concentrated by placing, drop by drop, on a small, sterile, unglazed porcelain plate until a fairly large accumulation of bacilli had been obtained. To these were added a few drops of 1:500, 1:5,000, and 1:50,000 sodium tellurite in distilled water, but no reduction was observed within forty-eight hours at 37 C.

Whether or not this test was really an index of viability of the tubercle bacillus was determined as follows: Two well-grown cultures of human tubercle bacilli on glycerol agar were exposed to the light of a 32 c. p. tungsten electric light in the incubator at 37 C., and several small lumps of these cultures were tested before and every twelve hours after exposure to the light by the tellurite drop test, and at the same time by inoculation into normal guinea-pigs. On the third day and later after exposure to the electric light, the lumps of culture gave a negative drop tellurite test, and, coincident with this, failed to produce tuberculosis in the guinea-pigs, whereas a lump taken from a control tube kept under the same conditions, but wrapped in heavy black paper to keep out the light, still reduced the tellurite and produced tuberculosis in guinea-pigs within seven days.

Belfanti suggested that the selenites and tellurites may be of value as a basis for a chemotherapy for tuberculosis. He did not, however, report any observations as a support for his statement. With a view to breaking ground in this direction, sodium tellurite was tested, so far as this was possible, for its value as a chemotherapeutic agent in tuberculosis. A few objections may immediately be made to its use; its ready reduction by the more active cells of the animal organism in comparison to its slow reduction by the rather inactive tubercle bacillus, and its chemical instability. Even these apparent disadvantages may become advantages under certain conditions, if, for instance, the

unreduced tellurite revealed a highly tuberculocidal action while practically non-lethal to the animal organism because of its ready power to reduce it. On account of the changes produced in the compound by introduction into the animal organism, the test for chemotherapeutic value naturally becomes more complicated. The following experiments are not conclusive, but can be used merely as an aid in carrying out further work with the selenites and tellurites.

As a gauge of the toxicity of a compound, such as, sodium tellurite, which is decomposed and reduced by the living tissues with which it comes in contact,³ it seems that the intravenous lethal dose is the only fair index for our purpose. For this reason, the toxicity of sodium tellurite was tested intravenously in rabbits with the results shown in Table 1.

TABLE 1
TOXICITY OF SODIUM TELLURITE INJECTED INTRAVENOUSLY IN RABBITS*

Rabbit	Weight of Animal in Grams	Amount Injected in Cubic-centimeters	Concentration of Sodium Tellurite Percentage	Amount Milligrams per Kilo	Result
1	1,350	5.0	0.01	0.37	Lived
1 Third day.	1.0	0.1	0.74	Lived
2	2,350	1.5	0.1	0.64	Lived
2 Fifth day..	2.0	0.1	0.85	Dead in 16 hours
3	2,350	2.0	0.1	0.85	Dead in 12 hours
4	1,450	1.5	0.1	1.03	Dead in 12 hours
5	1,150	2.0	0.1	1.74	Dead in 24 hours
6	1,130	2.0	0.1	1.77	Dead in 12 hours
7	1,250	1.0	1.0	8.00	Dead in 20 minutes

* In guinea-pigs (260-350 gm.), Gosio found that 10.0 mg. potassium tellurite, given subcutaneously in 5-10 c.c. distilled water or blood serum, were fatal in seven hours, 3 mg. in forty-eight hours, and 0.1-2.0 mg. produced only local necrosis and induration.

In experiments on mice, 0.2 mg. sodium tellurite intraperitoneally was found lethal to a 10 gm. mouse, whereas 0.02 and 0.04 mg. were not.

These experiments show that the intravenous lethal dose of sodium tellurite to rabbits is about 0.8 mg. per kilo body weight.

In order to determine whether sodium tellurite possessed any power to inhibit growth or had a tuberculocidal effect, a number of experiments were performed.

A series of glycerol agar tubes were made containing from 0.01-0.1 mg. sodium tellurite to 10 c.c. These were inoculated with human tubercle bacilli, placed in an incubator at 37 C., and observed at frequent intervals. After two months, all the tubes, controls, and tests revealed a good growth; the dilutions containing 0.05-0.1 mg. sodium tellurite revealed a definite reduction (blackening) of the original transplant and a dark gray color of the new growth, the edges being colorless in places, and the dilutions containing 0.01-0.05 mg. revealed a reduc-

tion (slight in the higher dilutions) by the original transplant, but the new growth was not even gray.

A series of tubes were made, each containing 2 c.c. of a uniform emulsion of human tubercle bacilli in 0.9 percent salt solution and varying amounts of sodium tellurite (0.01, 0.05, 0.1, and 0.2 mg.). These were placed in the incubator at 37 C. for forty-eight hours and then were injected into normal guinea-pigs. All the guinea-pigs developed tuberculosis.

As a result of these experiments, it can be stated that sodium tellurite, in amounts up to 0.1 mg. in 10 c.c. glycerol agar, does not inhibit or prevent the growth of the human tubercle bacillus; in amounts up to 0.2 mg. in 2 c.c. salt solution for forty-eight hours at 37 C., it does not kill the tubercle bacillus.

TABLE 2
EXPERIMENTS ON TUBERCULOCIDAL ACTION AND REDUCTION OF SODIUM TELLURITE

Concentration of Sodium Tellurite	Appearance of Culture Before Incubation	Results of Incubation	Result of Inoculation
0.1, A	Clear	Slight gray sediment	Dead 24 hours after injection
0.1, B	Turbid (ppt)	Heavy gray sediment	Dead 48 hours after injection
0.05, A	Clear	Slight gray sediment	Dead 24 hours after injection
0.05, B	Turbid (ppt)	Heavy gray sediment	Dead 24 hours after injection
0.01, A	Clear	Definite, slight gray sediment	Marked, generalized tuberculosis in 85 days
0.01, B	Clear	Fairly distinct, gray sediment	Marked, generalized tuberculosis in 48 days
0.005, A	Clear	Sediment, very slight gray	Marked, generalized tuberculosis in 85 days
0.005, B	Clear	Fairly distinct, gray sediment	Fairly advanced tuberculosis in 33 days
0.001, A	Clear	Very faint gray, indistinct sediment	Fairly advanced tuberculosis in 49 days
0.001, B	Clear	Fairly distinct, gray sediment	Well-advanced, generalized tuberculosis in 67 days
0.0001, A	Clear	Faint, questionable sediment	Well-advanced, generalized tuberculosis in 85 days
0.0001, B	Clear	Fairly distinct, dark gray sediment	Well-advanced, generalized tuberculosis in 85 days
Control 1, A	Clear	Faint gray sediment	Mild, generalized tuberculosis in 85 days
Control 2, A	Clear	Faint gray sediment	Fairly marked, generalized tuberculosis in 85 days
Control 1, B	Clear	Faint gray sediment	Marked, generalized tuberculosis in 55 days
Control 2, B	Clear	Faint gray sediment	Mild, generalized tuberculosis in 85 days

Thus far the tellurite has been tested only for its tuberculocidal action in fairly high dilutions, and the bactericidal action of the tellurite has been studied independent of whether it was reduced or not. In the hope of correlating these a little more, the following experiments were carried out. A much heavier emulsion of tubercle bacilli was used than had been used in most of the previous experiments, and one that should give an unquestionable reduction, if possible.

Two series of tubes were made, Series A containing 5 c.c. of 0.9 percent salt solution, and Series B, 5 c.c. of 5 percent glycerol broth, in which were suspended heavy emulsions of human tubercle bacilli, 2 mg. in each tube. To these were added varying amounts of sodium tellurite, making concentrations of 0.0001, 0.001, 0.005, 0.01, 0.05, and 0.1 percent. Two controls were made with 5 c.c. of 0.9 percent salt solution and two with 5 c.c. of 5 percent glycerol broth, containing a similar amount of emulsified human tubercle bacilli but without sodium tellurite. The tubes were all placed in the incubator at 37 C. for forty-eight hours, and injected into normal guinea-pigs at the end of this time. The results are given in Table 2.

GENERAL SUMMARY

As a result of an attempt to use the Gosio vital reaction (sodium tellurite) as an index of life of virulent human tubercle bacilli in bactericidal experiments in connection with chemotherapeutic work, it may be stated that it was not found to be an available general reagent for this purpose, at least by the methods tested.

Nevertheless, by its use a simple, rapid test was developed for determining the viability of cultures of tubercle bacilli, of value especially in eliminating such loss of time as may be occasioned by working with dead instead of viable cultures. A small lump of the culture to be tested is placed in the cup of a sterile, hollow glass slide and one or two small drops of sterile 0.2 percent sodium tellurite in distilled water are added; it is covered with a sterile glass cover slip bordered with sterile vaselin, and placed in the incubator at 37 C. Life of the organism is indicated by the blackening of the lump of culture, which occurs in from thirty minutes to two hours.

Sodium tellurite is lethal to rabbits when it is given intravenously in amounts of about 0.8 mg. per kilo. It does not kill the tubercle bacillus even when in 0.01 percent concentration in salt solution or glycerol broth for forty-eight hours at 37 C., nor does it inhibit the growth in 0.001 percent concentration on glycerol agar.

INOCULATION EXPERIMENT WITH PURE CULTURE OF SPIROCHAETA HYOS

STUDIES ON HOG-CHOLERA*

WALTER E. KING AND RAYMOND H. DRAKE

(From the Research Laboratory, Parke, Davis and Company, Detroit, Michigan)

In former publications it has been suggested that the spirochaeta hyos may bear some etiological relationship to hog-cholera. Heretofore, it has not been possible to prove the pathogenic significance of this new organism because of the difficulties encountered in attempting to obtain pure cultures. These difficulties have not been completely overcome as yet, but, by painstaking effort, a pure culture of the spirochaeta hyos has been secured and typical hog-cholera of the acute type has been produced with this culture. The protocol of this experiment is as follows:

On September 23, 1913, a culture was made on Hata medium with rabbit kidney from Berkefeld filtered suspension of tissue from a local lesion on the ear of Hog 653. The local ear lesion, on dark-field examination, showed numerous spirochetes. Hog 653, on autopsy, showed typical lesions of hog-cholera.

Culture 653, from the ear, was incubated in a desiccator, under anaerobic conditions, for several weeks at 40 C., and then for several weeks at 37 C. Dark-field examination, on December 13, showed the presence of spirochetes, relatively few in number. A portion of the impure culture was macerated in sterile water and filtered twice through the Berkefeld.

On January 5, 1914, cultures were made from the filtrate on Hata medium with no kidney tissue. Control cultures from the filtrate gave negative tests, showing that the filtrate was free from bacteria.

Culture 653, grown in the same manner as the above, was examined on March 17, on dark-field. It showed growth of the spirochaeta hyos in pure culture. Culture media, inoculated with material from Culture 653 (Transfer 1), gave negative results.

A suspension was made of a portion of pure culture of the spirochaeta hyos (Culture 653, Transfer 1) in an equal volume of sterile water. Dark-field examination showed the spirochaeta hyos, uncontaminated, in suspension. On March 17, animal inoculations were made from the suspension as follows: Hog 805, 4.5 c.c.; Hog 806, 3.5 c.c., both intramuscularly. A normal hog, Hog 807, was placed with them in an isolated, disinfected room as a control on Hogs 805 and 806.

Hog 805.—On March 17, Hog 805 was injected intramuscularly with 4.5 c.c. of Culture 653 (Transfer 1); March, 25, the hog appeared normal, appetite good,

* Received for publication October 14, 1914.

but somewhat inactive; March 27, normal in every way; April 6, "off feed," appeared sick; April 8, was bright; April 15, very sick; April 20, died.

Inguinal, mesenteric, retroperitoneal and other lymphatic glands were enlarged and very hemorrhagic; lungs, congested and consolidated; liver, mottled with areas of degeneration; spleen, much enlarged, dark and soft; kidneys, ecchymotic; intestinal mucosa, congested; no typical ulcers. *Spirochaeta hyos* present in cecal mucosa.

Hog 806.—On March 17, Hog 806 was injected intramuscularly with 3.5 c.c. of Culture 653 (Transfer 1); March 26, the hog was inactive, anorexia; March 27, acted better, appetite better; March 30, anorexia, weak, constipated; April 2, no appetite, back arched; April 6, numerous spirochetes in exudate from ear; April 8, very sick; April 13, moribund, killed.



Figure 1.—Hog 806

Lymphatic glands were very enlarged and hemorrhagic; liver, normal; lungs, congested and consolidated; spleen, soft and friable, enlarged; kidney, ecchymotic; small button ulcers in mucosa of large intestine. *Spirochaeta hyos* in mucosa of cecum.

Hog 807.—Hog 807 was a control on culture pigs. On March 17 the hog was placed with Hogs 805 and 806; April 1, normal, good appetite; April 2, slight symptoms; April 6, sick; April 7, anorexia; April 8, sick, constipated; April 15, very sick; April 17, died.

Lymphatic glands much enlarged and hemorrhagic; lungs, congested and consolidated; spleen, soft and friable, enlarged; kidneys, ecchymotic; small ulcers in mucosa of cecum, mucosa of large intestine congested. *Spirochaeta hyos* present in margins of cecal ulcers.

In this experiment Hog 805 showed a mild reaction eight days after inoculation and manifested "secondary" symptoms twenty days after

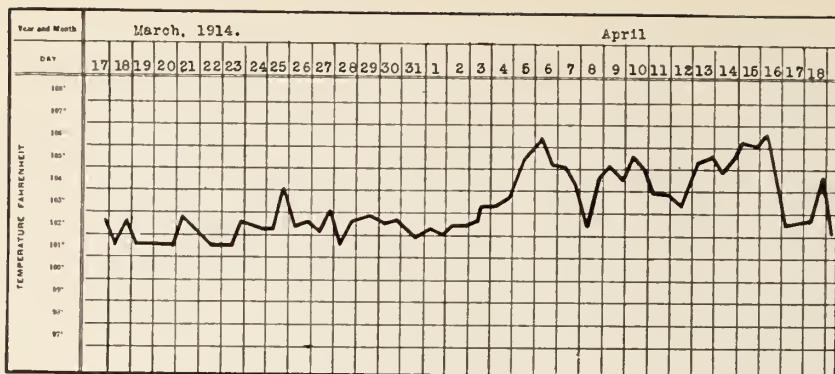


Chart 1.—Temperature Curve for Hog 805.

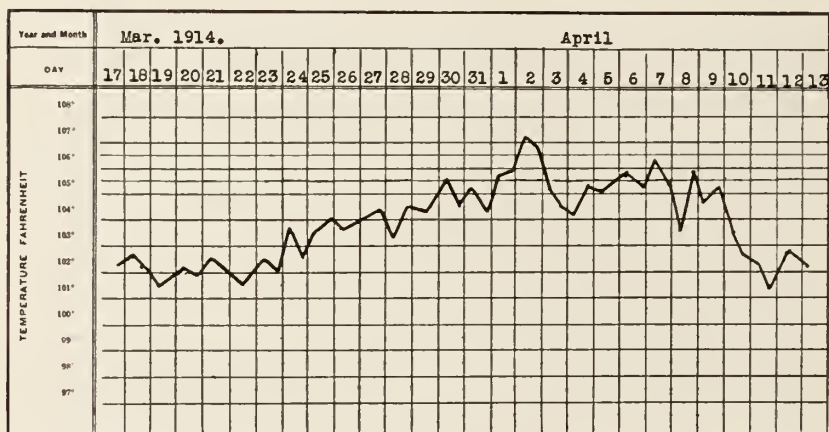


Chart 2.—Temperature Curve for Hog 806.

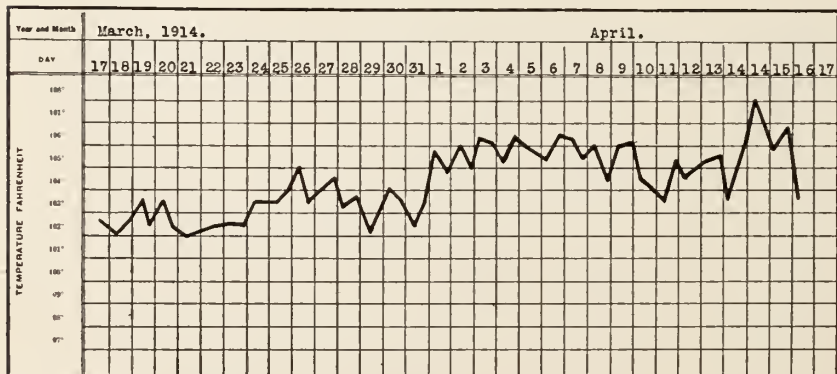


Chart 3.—Temperature Curve for Hog 807.

inoculation. This interesting phenomenon, which has been observed in some ten or twelve cases of hog-cholera produced by inoculating impure cultures of the spirochaeta hyos into healthy hogs, may represent the period of time necessary for certain stages of development of the spirochete to occur in the animal body.

Hog 806 acquired typical hog-cholera of the acute type from the inoculation with pure culture of the spirochaeta hyos, as controlled by the failure of symptoms to appear in the case of Check-Hog 807 until a sufficient time had elapsed for the control animal to acquire the disease by contagion.

The inoculation experiment should be repeated with other strains in pure culture before final conclusions are drawn. However, until substantial negative data can be presented by other investigators, the successful production of the disease with a pure culture of the spirochaeta hyos, together with other data already presented, justifies the statement: "Spirochaeta hyos is more nearly established as the specific cause of hog-cholera than any other known organism."

FURTHER OBSERVATIONS ON THE EFFECT OF QUININ IN RABIES*

V. H. MOON

(From the Memorial Institute for Infectious Diseases, Chicago)

In a previous paper¹ were given the results of an experiment in which dogs were inoculated with street virus and subsequently given large doses of quinin by mouth. Three dogs, so treated, lived, while the untreated controls died of rabies. The results were summarized as follows: "The above cases are not taken to mean that a cure for developed rabies has been found. It is known that in rabies, as in other diseases, there are variations in severity and virulence in different cases. It is possible that these cases were of sufficiently moderate severity that the quinin furnished barely sufficient aid to enable the system to throw off the disease, and that the same result would not have followed in severe cases. Viewed conservatively, the results are encouraging and indicate that the medical treatment of developed hydrophobia should not be regarded as hopeless. Should quinin not prove efficient when put to more severe tests, other agents should be given thorough trial experimentally."

Absence from laboratory facilities interrupted the further trial of quinin for several months. On resuming the tests, a strain of street virus was selected which was of somewhat greater virulence than that previously used. Street virus was used rather than fixed virus for the reason that it is the type responsible for practically all human cases of hydrophobia, and it was with a view to possible clinical application that the experiments were made. The technic of inoculation was the same as that previously used, i. e., anesthetized dogs were inoculated through the optic foramen by means of a long needle. Rabbits and guinea-pigs were inoculated intracranially through an opening made in the skull by a trocar. At varying periods following the inoculation, quinin was given in large doses either by mouth or by injection. In every case, an inoculated animal was kept untreated as a control. Quinin bisulphate was given by mouth, and, in a few instances, by injection. Otherwise, for both subcutaneous and intravenous admin-

* Received for publication October 13, 1914.

1. Jour. Infect. Dis., 1913, 13, p. 165.

istrations, the double hydrochlorid of quinin and urea was used. A brief summary of the experiments follows.

GROUP 1

October 31.—Rabbits 1 and 2 were inoculated.

November 15.—Rabbit 1, untreated, died. Negri bodies found in large numbers.

November 11.—Daily subcutaneous injections of 1 c.c. of a 10 percent solution of quinin bisulphate were begun on Rabbit 2. Dense areas of fibrosis and sloughs developed at the sites of injection.

November 21.—Immediate death was caused by 0.5 c.c. of the 10 percent solution, given intravenously. The rabbit had shown no signs of rabies, but Negri bodies were found in smear preparations from the hippocampus.

GROUP 2

November 5.—Rabbits 1 and 2 were inoculated.

November 20.—Rabbit 1, untreated, died. Negri bodies found.

November 9.—Daily subcutaneous injections of 1.5 c.c. of a 10 percent solution of quinin and urea begun on Rabbit 2. Indurations developed at the site of injections.

November 22.—An intravenous injection of 1 c.c. of the 10 percent solution of quinin and urea caused immediate death. The rabbit had shown no signs of rabies, and no Negri bodies were found.

GROUP 3

November 9.—Dogs 1, 2, and 3 inoculated.

November 24.—Dog 1 untreated, died. Negri bodies found. Rabies.

November 11.—Dogs 2 and 3 began receiving daily doses of 0.325 gm. quinin bisulphate by mouth.

November 26.—Dog 2 died. No symptom of rabies. Thick mucopurulent discharge from nose. Lungs consolidated in extensive areas. No Negri bodies found in hippocampus. Gram-positive diplococcus isolated from blood cultures. Guinea-pig, inoculated with substance of hippocampus, lived. Diagnosis, distemper.

December 4.—Dog 3 died with symptoms similar to those of Dog 2. The findings were also similar. Guinea-pig, inoculated with substance of hippocampus, lived. Diagnosis, distemper.

GROUP 4

December 8.—Dogs 1, 2, 3, and 4 inoculated.

January 8.—Dog 1, untreated, died. Combined symptoms of distemper and rabies. Negri bodies numerous. Gram-positive diplococcus cultivated from the blood. Guinea-pig, inoculated with brain, died of rabies. Diagnosis, rabies with distemper.

January 12.—Dog 2, treated since December 15 with quinin, 0.65 gm. daily, died after severe typical course of distemper. Distemper organism cultivated from blood and from bronchial exudate in consolidated lungs. No Negri bodies found. Rabbit, inoculated with brain substance, lived. Distemper. Dogs 3 and 4 died within two weeks following the inoculation. Distemper.

GROUP 5

January 9.—Dogs 1, 2, and 3 inoculated.

January 20.—Dog 1 died of meningitis on the tenth day after inoculation. No Negri bodies found.

January 24.—Dog 2, untreated, died after typical course of rabies. Negri bodies found.

January 28.—Dog 3 died after typical course of rabies. Had received 0.65 gm. of quinin daily since January 17. Negri bodies found. Rabbit, inoculated with brain, died of rabies.

GROUP 6

February 28.—Rabbits 1, 2, and 3 inoculated.

March 7.—Rabbit 1 given daily subcutaneous injections of 0.25 gm. quinin and urea.

March 11.—Indurations developed at site of injections. Given intravenous injection of 0.17 gm. daily.

March 21.—Died. Negri bodies found.

March 10.—Daily intravenous injections of 0.17 gm. begun on Rabbit 2.

March 15.—Died. Negri bodies found.

March 10.—Rabbit 3, untreated control, died. Negri bodies found.

GROUP 7

May 23.—Dogs 1, 2, and 3 were inoculated. Dogs 2 and 3 received 0.65 gm. quinin bisulphate by mouth daily beginning May 25.

June 22.—Dog 1, untreated, died. Negri bodies found in large numbers.

June 29.—Dog 2 died after a characteristic course of rabies. Negri bodies found.

June 15.—Dog 3 became very excitable, restless, and nervous and remained so for two weeks after which he gradually became normal.

June 25.—Guinea-pigs, inoculated intracranially June 18 with saliva of Dog 3, died of meningitis.

June 25.—Guinea-pigs, inoculated intramuscularly with saliva of Dog 3, lived.

QUININ IN HUMAN CASES OF HYDROPHOBIA

Harris² reported the successful treatment of a case clinically diagnosed as hydrophobia. The patient, who had been bitten several weeks before, presented symptoms which were sufficiently characteristic to warrant a clinical diagnosis of early hydrophobia. He recovered after intravenous administration of quinin and urea. Wesson³ and Williams⁴ each reports a case of hydrophobia in which quinin was given without success or apparent benefit.

I have had opportunity to treat two developed cases with quinin. The first case was a boy 5 years of age. On January 1, 1914, he was bitten on the face by a stray dog with which he and other children were playing. The dog was not captured and no suspicion of hydrophobia was aroused. On January 29 active symptoms developed, but the disease was not recognized until February 1, 9 a. m. At 3 p. m. when I first saw the boy, he was extremely restless, was able to swallow only with great difficulty, and showed classical hydrophobia and acrophobia. He was able to take 15 gr. quinin bisulphate in three doses by mouth after which he became unable to swallow and quinin and urea were given in

2. Jour. Amer. Med. Assn., 1913, 61, p. 1511.

3. Ibid., 1914, 62, p. 204.

4. Pub. Health Rep., 1914, 29, p. 949.

small doses intravenously and subcutaneously. Fifteen grains were given in the next six hours. The disease, meantime, was progressing rapidly and no effect from the quinin was evident. At 10 p. m., the child developed a severe acute mania on which sedatives had little effect. At 2:30 a. m., he gradually sank into a coma, and died at 12 m., February 2.

Case 2 was a colored woman, aged 60, who was bitten on the hand June 14. On July 24 she became sick, and, on July 27, the disease was recognized and she was brought to the hospital. The symptoms were not marked, and consisted of dizziness, slight difficulty in swallowing, restlessness, well-marked aerophobia, and spasmodic contractions of the muscles of respiration. She was given 20 gr. quinin bisulphate by mouth every two hours from 4 p. m., July 27, until 10 a. m., July 28, after which she became unable to swallow. In the afternoon two intravenous injections of 10 gr. each were given. No delay in the progress of the symptoms was noticed. At 5 p. m., she developed convulsions and acute mania, making restraint necessary. This condition progressed steadily during the night. Death occurred at 7 a. m., July 29.

These cases illustrate the difficulty of applying any measures in the early stages of developed hydrophobia. The disease is not recognized when its symptoms first appear, and when recognized the termination is usually near at hand.

DISCUSSION

In Group 1, the treated rabbit lived six days longer than the control, and died as a result of too large an intravenous injection of quinin. However, Negri bodies were found in the brain which indicates that the treatment had not prevented the disease from developing.

Group 2 was similar in results to Group 1, except that no Negri bodies were found in the brain of the treated rabbit. In both of these groups, the subcutaneous injection of quinin produced dense indurations and necrosis which caused the discontinuation of further treatment by this method. The danger of death, following intravenous injections of quinin in rabbits, and the difficulty of administering quinin by mouth led me to use dogs in the following groups.

In Groups 3, 4, and 6, clear-cut results were prevented by the prevalence for months of a severe form of distemper in the kennels. Repeated disinfections were ineffective in preventing the recurrence of the malady in successive groups. These groups are inconclusive as to the curative effect of quinin, but they are of sufficient significance to warrant their consideration here.

In Group 3, Dogs 2 and 3, quinin treated, died two days and ten days, respectively, later than their untreated controls.

In Group 4, the quinin treated dog died four days later than the control.

In Group 5, one dog, untreated, died on the tenth day from infection of meninges probably introduced at the time of inoculation. The remaining two dogs of the group did not have distemper. The treated dog died four days later than the control, and both died of rabies. This represents a clear-cut failure of quinin to cure or to prevent the development of rabies in dogs.

In Group 6, rabbits were again used and the quinin, in the form of double hydrochlorid of quinin and urea, was given intravenously. The treated rabbits died of rabies five and eleven days, respectively, later than the controls. Again, the treatment neither prevented nor cured the disease.

In Group 7, one treated dog died of rabies seven days later than the control. The other treated dog lived, but the evidence is not conclusive whether the inoculation failed to produce the disease, whether this was one of the so-called abortive cases, or whether the treatment had something to do with the result.

Assuming that each of the treated dogs in Groups 3 and 4 had died of rabies, instead of distemper, the fact remains that, in no case, did the treated animal die as early as the control. If any significance is to be attached to this it would point to the possible application of quinin in human cases when, for any reason, the Pasteur treatment is begun late and a prolongation of the incubation period would give time for the completion of the preventive immunization.

Cummings⁵ and Frothingham and Halliday⁶ report the unsuccessful treatment of rabbits and guinea-pigs by subcutaneous and intravenous injections of quinin.

CONCLUSIONS

Quinin has failed to be regularly effective as a cure or preventive of rabies in animals.

Quinin, given in the latter stages of hydrophobia in two human cases, produced no significant results.

Quinin appears to retard somewhat the development of street rabies if given in large doses during the incubation period. The results indicate that the organism, which causes rabies, is influenced in some degree by quinin. This is significant as showing that the organism is susceptible to therapeutic measures, and gives reason to hope that some drug may be found which will be of value in the treatment of hydrophobia.

5. *Jour. Infect. Dis.*, 1914, 15, p. 209.

6. *Jour. Med. Research*, 1914, 30, p. 275.

ON THE SPECIFIC PRECIPITIN IN THE BLOOD OF PERSONS INJECTED WITH ANTIDIPHThERIC HORSE SERUM*

CLIFFORD W. WELLS

(From the Memorial Institute for Infectious Diseases, Chicago)

Kraus,¹ in 1897, first demonstrated that the serum of an animal immunized against a foreign serum, when mixed with the immunizing serum, gives rise to a precipitate. A few years later, Hamburger and Moro,² v. Pirquet,³ and v. Dungern⁴ demonstrated in the serum of diphtheria patients, who had received antitoxic horse serum, the presence of a precipitin for horse serum, normal or antitoxic.

The method I have used in the study of this precipitin consisted in the macroscopical titration of serum, twenty-four hours old, from diphtheria patients, who had received therapeutic doses of antitoxic serum, against normal or antitoxic horse serum. In most tests, dilutions were made of the antigen, or horse serum, while the human serum was used undiluted; when the horse serum was undiluted and the human serum diluted, no marked variation in the results was obtained.

In order to economize on serum, small tubes with a lumen of about 3 mm. were employed. These were made by sealing in a Bunsen flame one end of thick glass tubes, about 6 cm. long. The thickness of the glass served somewhat to magnify the precipitin ring at the point of contact of the two sera. The antigens, consisting in majority of cases of normal horse serum, was first introduced into the tubes in various dilutions and they were arranged in a row in special racks, the depth being about 1 cm. Then, by means of fine pipettes, the human serum, in about equal amount, was run carefully into the bottom of the tubes in such a way that a definite line of contact was produced. The tubes were incubated at 37 C. for two hours, at the end of which time the results were noted.

At first, some confusion was encountered in the readings, because frequently the human serum was turbid, rendering the detection of a

* Received for publication November 3, 1914.

1. Wien. klin. Wehnschr., 1897, 10, p. 736.

2. Ibid., 1903, 15, p. 445.

3. Die Serumkrankheit, 1905, Vienna.

4. Die Antikörper, 1903, Jena.

precipitin ring difficult. It was found that dilution of the human serum with equal parts of salt solution increased the accuracy of the readings in such cases. McGowan⁵ has suggested that turbidity may be due to a lipemic condition, an anemic or an emaciated state. One or all these conditions frequently existed in my cases; the lipemic condition, because of the large amounts of milk and cream given the patients; the other states were results of the disease itself.

ANALYSIS OF PRECIPITIN CURVES

Welch and Chapman⁶ have advanced the idea that the precipitate is formed by constituents of the antigen and that there is a close rela-

TABLE 1
ANALYSES OF TWENTY-SIX CASES AS TO THE QUANTITY OF PRECIPITIN IN RELATION TO THE AMOUNT AND LOT OF ANTITOXIN SERUM USED

Case	Age of Patient	Lot of Antitoxic Serum	Units of Antitoxin	Cubic Centimeters of Serum Injected	Titer of Precipitin as Measured by Dilution of Antigen	Number of Days Between Last Injection of Serum and Appearance of Precipitin
634	25	S6	10,000	9	0	9
638	25	S8	10,000	7	0	6
641	6	S6	10,000	9	5	11
647	9	S8	30,000	21	10	9
628	23	440	40,000	27	20	5
630	40	S6	15,000	13.5	20	6
646	21	S8	10,000	7	20	11
637	6	S6	40,000	36	40	6
670	8	S6	20,000	18	40	9
624	5	S8	10,000	7	80	4
649	14	S8	5,000	3.5	80	8
651	10	S8	40,000	28	160	7
611	28	440	10,000	6.5	1,280	6
588	13	S6	35,000	31	1,280	7
570	37	S6	20,000	18	1,280	5
618	25	440	15,000	10	1,280	?
653	45	S8	10,000	7	2,560	5
597	7	S6	30,000	27	2,560	4
572	24	S6	15,000	13.5	2,560	7
599	16	440	35,000	24	5,120	7
556	18	S6	20,000	18	10,240	7
547	19	S6	25,000	22.5	20,480	7
591	22	S6	15,000	13.5	20,480	5
617	18	440	60,000	42	20,480	5
535	22	S6	40,000	36	20,480	8
578	8	S6	35,000	31	40,960	8

tionship between the quantity of the precipitate and the amount of antigen injected. Table 1, giving an analysis of twenty-six cases, demonstrates conclusively that the amount of precipitin present, as determined by the titer of the antigen, in which when mixed with human serum the precipitate appeared, is not dependable upon the

5. Jour. Path. and Bacteriol., 1909-10, 14, p. 395.

6. Brit. Med. Jour., 1910, 2, p. 1510.

amount of antitoxic serum injected, nor does there seem to be any relation between the amount of precipitin and the various lots of antitoxic serum used. In all cases the antitoxic serum was so-called concentrated or precipitated serum.

A study of the respective ages, sexes, and weights of the patients shows that these factors have little, if any, bearing on the amount of precipitin present. Thus, we are led to the conclusion that the precipitin content of the patient's serum is dependable upon intrinsic elements or processes of the patient's organism, the exact nature of which we, as yet, do not understand; and that it depends on the antitoxic serum only in that this substance, which consists of foreign protein, initiates or stimulates the formation, by the body cells, of that substance, which, when brought in contact with the antidiphtheric serum or normal horse serum, produces a precipitate. It is justifiable to infer that the blood serum of those patients, who showed the presence of precipitin in very high dilutions of antigen, as in Cases 535, 578, and 617 (Charts 1 and 2) who received 36, 31, and 42 c.c. of serum, respectively, would have shown a marked precipitin production following injections of much smaller quantities of antitoxic serum. From a study of serum disease in diphtheria, there has been noted a marked relationship between certain lots of antitoxic serum and the occurrence of the serum disease, but in respect to any relationship between the lot of antitoxic serum and the amount of precipitin produced in the patient's serum no relationship has been noted (Table 1).

Closely related to the foregoing point is the relationship between the duration of precipitin in the patient's serum and the amount and lot of antitoxic serum administered. Table 2 presents an analysis of twenty-three cases with special reference to this point. From a consideration of this table, it appears that the amount or lot of antitoxic serum has little, if any, influence upon the duration of precipitin for horse serum in the blood of the patients. Cases 647 and 646 demonstrate that, in certain individuals, a small dose of the same lot of antitoxic serum may give a longer appearance of precipitin than a larger dose. Case 647 received 30,000 units, or 21 c.c., of Lot S8 and the precipitin appeared for fourteen days; Case 646 received 10,000 units, or 7 c.c., of the same lot, and the precipitin appeared for seventeen days; Case 649, which received only 5,000 units, or 3.5 c.c., of the same lot, showed precipitin as late as the thirty-sixth day, when the patient was discharged; Case 628 received 40,000 units, or 27 c.c., of Lot 440 and showed precipitin only eight days, while Case 618, receiving 15,000

units, or 10 c.c., of the same lot, showed a high content of precipitin on the fifty-fourth day, when discharged (Chart 1).

An explanation, therefore, of the cause of the duration of precipitin in the blood rests, as does the explanation of the quantity of precipitin, upon certain intrinsic properties of the organism, the exact nature of which we do not as yet understand. At this point it is of interest to note that Hektoen⁷ has shown that the amount of antitoxic serum injected does not bear a definite relation to the amount of specific agglutinin, lysin, and opsonin produced.

TABLE 2
ANALYSES OF TWENTY-THREE CASES AS TO THE RELATION OF LOT AND AMOUNT OF ANTITOXIC SERUM TO INCUBATION PERIOD AND DURATION OF PRECIPITIN IN BLOOD OF PATIENTS

Case	Age of Patient	Lot of Antitoxic Serum	Units and Quantity of Antitoxin Injected		Number of Days Between Last Injection of Serum and Appearance of Precipitin	Duration in Days of Precipitin in Blood
628	23	440	40,000	27 c.c.	9	8
630	40	S6	15,000	13.5 c.c.	6	9+
634	25	S6	10,000	9 c.c.	9	11
647	9	S8	30,000	21 c.c.	9	14
597	7	S6	30,000	27 c.c.	4	17
646	21	S8	10,000	7 c.c.	11	17
637	6	S6	40,000	36 c.c.	6	18+
611	28	440	10,000	6.5 c.c.	6	19+
588	13	S6	35,000	31 c.c.	7	21+
641	6	S6	10,000	9 c.c.	11	21+
570	37	S6	20,000	18 c.c.	5	22
547	19	S6	25,000	22.5 c.c.	9	23+
572	24	S6	15,000	13.5 c.c.	7	26
578	8	S6	35,000	31 c.c.	8	28+
624	5	S8	10,000	7 c.c.	4	30
599	16	440	35,000	24 c.c.	7	31
591	22	S6	15,000	13.5 c.c.	5	33+
651	10	S8	40,000	28 c.c.	7	35+
649	14	S8	5,000	3.5 c.c.	8	36+
535	22	S6	40,000	36 c.c.	8	43+
617	18	440	60,000	42 c.c.	5	46+
536	18	S6	20,000	18 c.c.	7	53
618	25	440	15,000	10 c.c.	?	54+

Plus sign following the number of days signifies that precipitin was present at the time of discharge from hospital.

Average number of days per case: 26.

Average number of days for each lot: Lot S6, 23.9; Lot S8, 25; Lot 440, 31.6.

Von Pirquet³ suggests that the variability of the incubation, or latent period, may possibly be due to the amount or method of injection of the antigen. The results of this study, however, fail to show any connection of importance between the amount of antigen injected and the length of the incubation period (Table 2).

Case 630, receiving 15,000 units, or 13.5 c.c., of Lot S6, had an incubation period of six days; Case 637, receiving 40,000 units, or 36 c.c., of the same lot, also had an incubation period of six days; Case 570, receiving 20,000 units, or 18 c.c., of the same lot, had an incubation period of five days; Case 535, receiving 40,000 units, or 36 c.c., of the same lot, had an incubation period of eight days. A study of cases receiving Lot S8 shows a similar relationship.

The manner of administration of the antigen is of little value in this series, as in practically every instance the antitoxin was administered intramuscularly.

Charts 1 and 2 show more or less typical precipitin curves from the cases included in this study. The curves in Chart 2 show a feeble

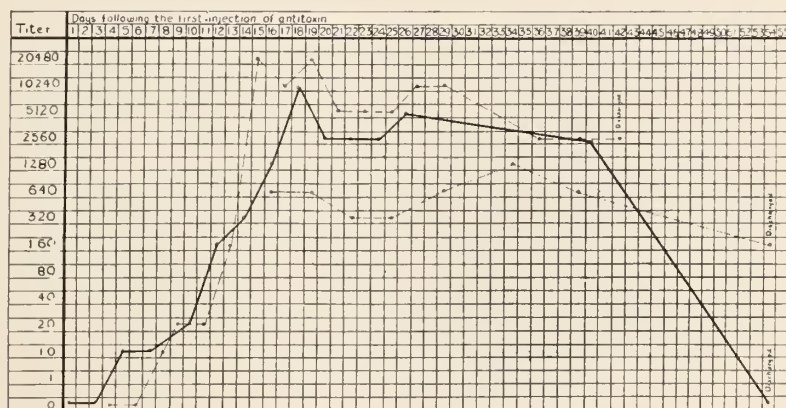


Chart 1.—Typical precipitin curves showing different phases. Small circles show the days on which the blood was withdrawn for titration.

Case 536 (heavy, solid line).—Male, age 18 years. Ill four days before receiving antitoxin, which was administered on three successive days, totaling 20,000 units, or 18 c.c., of Lot S6. No serum reaction was noted.

Case 535 (broken line).—Male, age 22 years. Ill three days before receiving antitoxin, which was administered on three successive days, amounting altogether to 40,000 units, or 36 c.c., of Lot S6. No serum reaction was noted. Complicated by typhoid fever.

Case 618 (dotted line).—Female, age 25 years. Received antitoxin on the day following the onset of diphtheria in one dose of 15,000 units, or 10 c.c., of Lot 440. Serum was not examined until the sixteenth day.

precipitin reaction in respect to quantity, but a strong reaction in respect to duration. Those in Chart 1 show a more marked reaction in both respects.

Von Dungern⁴ differentiates four phases in a typical precipitin curve. These phases are fairly well differentiated in the curves presented in the charts: Phase 1, or latent period, in twenty-five cases averaged 6.7 days, varying from 3-12 days; Phase 2, or ascending

phase of precipitin content, in eighteen cases averaged 5.1 days, varying from 3-15 days; Phase 3, or antibody plateau, in seventeen cases averaged 16.3 days; Phase 4, or decline of antibodies, could not be followed to the end in all my cases because of the discharge of the patients from the hospital before this point was reached. In a few cases, however, where it was possible to follow the precipitin curve to a completion, this phase was found to be short, averaging from 3-6 days.

Von Dungern's differentiation was made with precipitin in rabbit serum, consequently a close comparison of the duration of each phase with precipitin in human serum is of little value. However, the time in each case varies but little. Von Dungern's time for each phase was as follows: Phase 1, 4.5-5.5 days; Phase 2, about 2 days; Phase 3, variable, 3-9 weeks; Phase 4, variable, usually a few days.

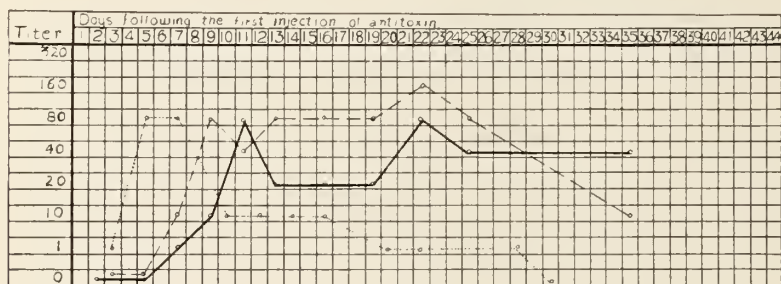


Chart 2.—Showing low precipitin content with typical curves. Small circles designate days on which blood was withdrawn for examination.

Case 649 (heavy, solid line).—Male, age 14 years. Antitoxin amounting to 6,000 units, or 3.5 c.c., of Lot S8 was given seven days after the onset of the disease. No serum reaction.

Case 651 (broken line).—Male, age 10 years. He was given 40,000 units of antitoxin, or 28 c.c., of Lot S8 four days after the onset of the disease. Urticaria appeared on the third day after the antitoxin was given and disappeared on the eighth day.

Case 624 (dotted line).—Male, age 5 years. Antitoxin amounting to 10,000 units, or 7 c.c., of Lot S8 was given three days after the onset of the disease. No serum reaction occurred.

It may be noted that, on the whole, the precipitin curves in my cases follow very closely the typical antibody curves, and especially do they follow the curves obtained by Hektoen⁷ for opsonin, agglutination and lysin for horse corpuscles in blood of man injected with antidiphtheric horse serum.

SERUM DISEASE

Hamburger and Moro² suggest a fundamental connection between the appearance of precipitin in the blood of diphtheria patients, who have received antitoxic serum, and the appearance of serum disease.

While they reject several theories which have been entertained concerning the etiology and genesis of the serum disease, no satisfactory explanation is advanced by them.

A number of cases in this study presented a definite precipitin curve and, at the same time, typical cutaneous serum reactions. From a study of the course of the serum reactions in these cases and its effect on the precipitin curve, there is some indication of a connection or a relationship between the serum disease and the precipitin content of the patient's blood.

Of the twenty-seven cases, nine manifested a recognizable serum disease. Analyses of some of these cases are interesting.

Case 617.—The precipitin curve shown in Chart 3 demonstrates a certain connection between the serum disease and the precipitin content of the serum. On the seventh day following the injection of anti-toxic serum, while precipitin was demonstrable in a dilution of 640 of the antigen, urticaria of a typical form developed with other recognized signs of serum disease which continued until the thirteenth day. In the midst of this intervening period there occurred on the tenth day a marked fall in the precipitin content to a dilution of 80 of the antigen, which fall was followed on the thirteenth day, the day on which the urticaria faded, by a sudden rise in the precipitin to a dilution of 20,480 of the antigen, which dropped on the fourteenth day to a dilution of 1,280, where it remained constant for a number of days.

Cases 599 and 578 (Chart 3) and Case 651 (Chart 2).—These cases demonstrate the same general connection, except that in the curves of these cases there is not present a sudden or marked drop in precipitin content with the onset or during the course of the serum disease, but there is shown a marked rise in each precipitin curve synchronous with the disappearance of the serum disease.

Case 646.—The precipitin curve of this case is not recorded on the charts, as the patient developed urticaria which faded before precipitin was demonstrable in the patient's serum.

From the observations in these five cases, we are warranted in drawing the conclusion that the serum reaction is connected with a union of the precipitin element with some other element in some way not apparent or understood, and that the disappearance of the urticaria and other symptoms of serum disease is synchronous with the liberation of the precipitin from this union, resulting in a sudden rise in the precipitin content of the blood. The extent of the fall in the pre-

cipitin content at the onset of the serum disease would seem to be dependent on the rapidity of precipitin formation compared to the rapidity of its union or consumption for the production of the phenomenon of the serum disease.

By some it has been intimated that the visible manifestations of serum reaction, and particularly the cutaneous manifestations, are the result of an actual precipitation in the blood, which results in the plugging of small capillaries; such precipitation would also result in the removal of a certain amount of demonstrable precipitin from the free,

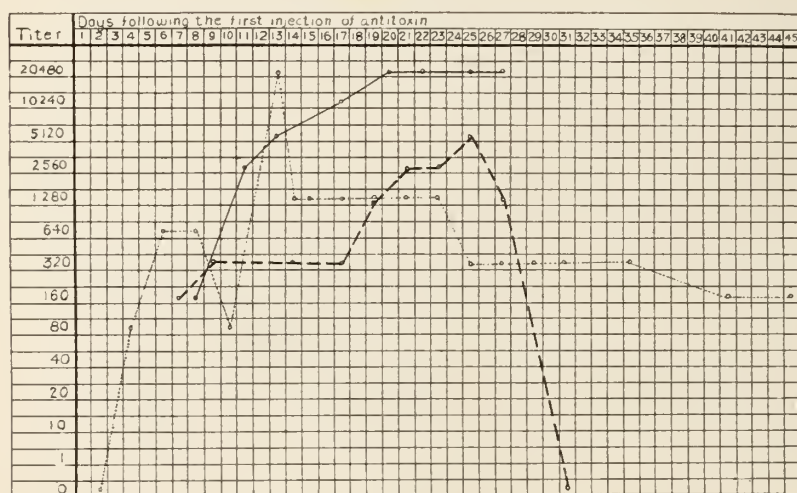


Chart 3.—The effect of serum reaction on precipitin content in the blood serum, also atypical precipitin plateaus.

Case 578 (heavy, solid line).—Male, age 8 years. First received antitoxic serum on day following onset of disease, altogether 35,000 units, or 3 c.c., of Lot S6 were given. Serum reaction, as manifested by urticaria, appeared on the third day, and disappeared on the seventh day.

Case 599 (broken line).—Female, age 16 years. Antitoxic serum first administered on the day of the onset of the disease and additional doses were given on three succeeding days, until a total of 35,000 units, or 24 c.c., of Lot 449 was given. Serum reaction, consisting of urticaria, appeared on the tenth day and disappeared on the fourteenth day.

Case 617 (dotted line).—Female, age 18 years. Antitoxic serum first given two days following onset of disease. Additional doses given on two successive days, until a total of 61,000 units, or 42 c.c., of Lot 440 was given. Serum reaction appeared on the seventh day and disappeared on the thirteenth day.

circulating blood. Whether or not this is the explanation of the apparent relationship between the precipitin content and the serum reaction has not been settled in this study, but late observations on this point by Hamburger and Moro² have seemed to disprove the possibility of a plugging of capillaries with a demonstrable precipitation in the blood.

While no special study of the phenomenon was undertaken in this series of cases, it is interesting to note that nine of twenty-seven cases showed a serum reaction; and that in these nine cases the incubation, or latent period averaged 9.75 days, which agrees closely with the results obtained by Weaver,⁸ who found an incubation period of 8.4 days in 801 cases of serum disease, and Cowie,⁹ who found an average of 5-7 days.

SUMMARY

The amount of the precipitin, measured by the degree of dilution of the antigen in which the precipitin is demonstrable, is not dependent on the amount or lot of antitoxic serum administered, nor on the age, sex, or weight, of the patient. Neither is the duration in days of precipitin in the patient's serum dependent on any of these factors.

The amount and possibly the duration of precipitin in the blood are dependent on some, as yet unknown, intrinsic process of the organism, which, however, is initiated or activated by the introduction of foreign protein in the antitoxic serum.

There is no evidence in this series to indicate that the length of the incubation, or latent period of the precipitin element is influenced materially by the quantity or method of administration of the antitoxic serum.

The various phases of the precipitin curve in this series compare favorably with the length of the respective phases of the antibody curves.

Precipitin is utilized or withdrawn from the blood during the course of the serum disease, and possibly is a factor in the production of the various phenomena of serum disease.

8. Arch. Int. Med., 1909, 3, p. 485.

9. Am. Jour. Dis. Child., 1914, 7, p. 253.

THE FECAL FLORA OF TYPHOID FEVER AND ITS REACTION TO VARIOUS DIETS*

JOHN C. TORREY

(Department of Experimental Pathology, Loomis Laboratory, Cornell University Medical College, New York)

Altho the typhoid bacillus itself has been the subject of many investigations and much is known in regard to its biology and its pathogenesis, little exact information has been acquired in reference to the intestinal bacterial conditions which may be associated with an invasion by this bacillus. To be sure, typhoid fever is no longer to be regarded as essentially an intestinal disease, yet bacterial conditions in the intestines may be conceived to be such, in certain cases, as to add the weight of the toxic products of putrefaction to the human organism already oppressed by the typhoidal virus; in other patients, the fecal flora may be of a type which permits the most favorable digestion and absorption of the food administered.

As is well known, Coleman,¹ for a number of years, has been placing his typhoid patients on a high calory diet in order to reduce to a minimum the severe loss of nitrogen and weight which usually occurs in such cases when kept on a low calory diet. In connection with these cases in addition to careful clinical histories, a very accurate record is kept of the amounts of carbohydrate, fat, and protein consumed daily by each patient. The conditions are, accordingly, such as to present a most favorable opportunity to study the dietetic response of the typhoidal intestinal flora, and through the courtesy and cooperation of Dr. Warren Coleman the material for this study has been available.

As far as I am aware, this investigation is the first in which an attempt has been made to correlate conditions and changes in the fecal flora of human subjects with exact data in regard to the constitution of the diet utilized. It seems not unlikely that information of this character may give a clue to the most favorable dietaries for various infections, especially those involving the digestive tract. As Herter² has well suggested in connection with a general discussion of intestinal

* Received for publication November 6, 1914.

1. Schaffer and Coleman: Arch. Int. Med., 1909, 4, p. 538.

2. Bacterial Infections of the Digestive Tract, New York, 1907, p. 181.

infections, "Our attention has, perhaps, been too exclusively fixed on the specific excitants, and the rôle played by associated bacteria must receive more study for it is clear that they sometimes play a significant part in determining the outcome of an infection. The difference that decides whether a man will live or die must frequently be a slight one looked at from the standpoint of the processes of battle within the body."

It has been repeatedly observed that a diet containing large amounts of carbohydrate not only encourages the development of a fermentative flora, but also, as Kendall³ has recently pointed out, tends to protect the protein in the food from putrefactive decomposition, not alone by the obligate putrefactive bacteria, but also by members of the colon and proteus groups capable of exercising either putrefactive or fermentative activities. Altho much laboratory evidence has been advanced in support of this principle, little definite information has been adduced in regard to the amount of carbohydrate which should be incorporated in the diet of adults in order to bring about this result or in regard to the uniformity and degree to which this is effected in individuals with various types of fecal flora.

In this study, the aim has been to determine the biological propensities and activities of the fecal flora as a whole, rather than to isolate and classify individual bacteria which may happen to be present in the stools and in regard to numbers and significance of which there is no definite criterion. In order to effect this purpose, the methods introduced by Herter⁴ and elaborated by Herter and Kendall,⁵ in their feeding experiments with monkeys, and by MacNeal, Latzer, and Kerr,⁶ in a study of the normal intestinal flora of man, have been adopted with some modifications. Altho the determination of the general type of flora has been the primary objective, the identification of bacterial types has always been effected when it would seem to serve a useful purpose.

This investigation was carried out during a part of the years 1912-1913.

During this period, a total of over one hundred stools from twenty-two typhoid patients have been examined. In a few instances, only a single specimen from a patient was subjected to the various procedures, but in twelve

3. Jour. Med. Research, 1911, 25, p. 117; Kendall and Farmer: Jour. Biol. Chem., 1912-13, 13, p. 63.

4. Bacterial Infections of the Digestive Tract, New York, 1907, p. 181.

5. Jour. Biol. Chem., 1909-10, 7, p. 203.

6. Jour. Infect. Dis., 1909, 6, pp. 123, 571.

cases a series of four to seventeen stools were investigated. With a few exceptions, these patients were from the metabolism ward of the second medical division of Bellevue Hospital. After October 1, 1913, the Russell Sage Institute of Pathology was in charge of this ward. It is a pleasure to acknowledge the assistance of Dr. Eugene F. DuBois and others of the Russell Sage Institute. I also wish to thank Dr. N. M. Keith of the New York Hospital for his aid.

METHODS

The material collected for examination was generally a part of a movement occurring between 6 and 7 o'clock in the morning. The stool was received in a sterile bed pan and a part transferred to a sterile bottle. These specimens were kept on ice during the few hours which might elapse before examination. As a number of the patients were inclined to constipation, it was frequently necessary to use an enema, consisting generally of a sterile normal saline solution.

With all the specimens, except those from fluid stools, a definite weighed amount of feces was used in seeding the various culture media. In making the dilutions of the feces, a method similar to that of MacNeal⁷ was followed, viz., 500 mg. of fecal matter, representative of the whole specimen as regards moisture and consistency, were carefully weighed in a large sterile watch glass. This material was then thoroughly emulsified in 50 c.c. of normal salt solution and poured into a small flask. Each cubic centimeter, accordingly, held the equivalent of 10 mg. of the fecal matter. With fluid stools, an emulsion was made to the same density as one of these weighed preparations. From this primary emulsion, smears were made and treated with gram stain for microscopical examination and differential count.

A series of dilutions of the fecal emulsion at 1-10, 1-100, 1-1,000, and 1-10,000 were next prepared and from suitable dilutions, using 1 c.c. in each case, poured plates were made with sugar-free agar, 1 percent lactose agar, and standard nutrient gelatin. These media were all titrated + 1.0 to phenolphthalein. The sugar-free agar plate was incubated aerobically and the lactose agar plate anaerobically at 37 C. Definite amounts of the diluted material were also spread on large Endo plates with sterile glass rods. The Endo medium was prepared in accordance with Kendall and Day's⁷ modification. After twenty-four hours' incubation, typhoid-like colonies on the Endo plates were transferred to Russell's⁸ double sugar medium and characteristic growths were subjected to agglutination and cultural tests.

The following media were seeded with 0.5 c.c. of the primary fecal emulsion: Fermentation tubes containing sugar-free meat infusion peptone broth plus 1 percent dextrose, lactose, and saccharose; a fermentation tube containing litmus milk; and tubes containing N/20, N/10, and N/5 acetic acid dextrose broth. This acetic acid medium was prepared in the following manner: Acetic acid was added to 1 percent dextrose meat infusion peptone broth until the reaction became N/5 acid to phenolphthalein. Part of this was then diluted with neutral dextrose broth until the N/10 and N/20 titrations were obtained. The three lots of this medium were tubed in 10 c.c. amounts and plugged with paraffined cotton. These tubes were incubated at 37 C.

The undiluted feces were used in seeding the following media: About 0.5 gm. was emulsified in the open arm of a large fermentation tube containing lactose peptone bile; a loop was streaked on a slant of Loeffler's serum medium;

7. Jour. Med. Research, 1911, 25, p. 95.

8. Ibid., p. 217.

and a stab culture was made in nutrient gelatin. The bile and serum media were incubated at 37 C. and the gelatin was incubated at room temperature.

About 10 c.c. of a 1-10 dilution of the original fecal emulsion were heated at 80 C. for fifteen minutes for spore cultures. One cubic centimeter was plated in sugar-free agar and in lactose agar, and also, in some instances, the same amount was added to fermentation tubes, containing milk with defibrinated rabbit or dog blood added, and to lactose blood-agar plates. These cultures were incubated at 37 C.

All the plate cultures containing lactose were incubated at 37 C. under anaerobic conditions. The method of Zinsser⁹ was followed in making these anaerobic plate cultures and proved entirely satisfactory. To insure a higher degree of anaerobiosis and to protect the surfaces of the seeded plates from the pyrogallic sodium hydrate solution, the hardened inoculated medium was covered with a layer of sterile agar.

After twenty-four hours' incubation, the Endo plates, the sugar-free agar plates, and the fermentation tubes were examined and the results recorded. After forty-eight to seventy-two hours, the examination included the gelatin and the anaerobic plates, and also the acetic acid cultures. The spore cultures were incubated several days.

The gram stain, used in coloring the smears from the fecal emulsion and the sediments of the fermentation tubes, was prepared and employed as recommended by Elser and Huntoon.¹⁰ The staining procedures were similar in each instance, except that the period of decolorization with absolute alcohol was varied in accordance with the thickness of the smear. In a number of instances, the results with this method were compared with results from the use of preparations decolorized with xylol and anilin oil followed by methyl alcohol, as recommended by MacNeal for work of this character. Sharper differentiation, however, was obtained by the treatment with absolute alcohol. A total of 300 bacteria was counted from each specimen and the results expressed in percentages. I am indebted to Mr. A. H. Rahe, my assistant, for many differential bacterial counts.

SIGNIFICANCE OF THE VARIOUS TESTS

The several procedures followed in these examinations were selected because, taken as a whole, they seemed to give a composite picture of the character of the stool and, through them, changes in the fecal flora following modifications in the diet could be satisfactorily followed. The general purpose of each test is as follows:

Endo Plates.—The primary object in the use of these plates was to gain an insight into the general character of the viable aerobic flora of the stool. With a little experience, it is possible from a mere inspection of these plates to identify a considerable number of different types of bacterial colonies, such as *B. coli*, *B. lactis aerogenes*, *B. alkaligenes*, the colon types which do not act on lactose and which for convenience are designated *B. paracoli* in the tables, also *B. pyocyaneus*, *B. proteus*, streptococcus, and staphylococcus. In the tables, the relative number of colonies appearing on these plates is indicated by the number of

9. Jour. Exper. Med., 1906, 8, p. 542.

10. Jour. Med. Research, 1909, 20, p. 377.

crosses, and the most numerous types of bacterial colonies are written below in order of prevalence. A search was always made for typhoid colonies and, as two or more plates with a favorable distribution of colonies were almost always obtained, viz., 250-300 colonies on a plate, the failure to find any typhoid colonies indicated that these bacilli were present in the feces in small numbers, if at all. Ficker and Hoffmann¹¹ estimated that, with the Drigalski plate medium, the isolation of typhoid bacilli from stools was unlikely in cases where the viable fecal bacteria outnumbered the typhoid bacilli more than 300 to 1. In this series of patients on the high calory diet, the typhoid bacillus was isolated from the stools infrequently either from directly seeded Endo plates or from bile enrichment tubes.



Fig. 1.—Lactose agar anaerobic plating, showing the grouping of the aciduric bacteria colony about a central colon colony. X1.

Sugar-Free Agar Aerobic Plates and Lactose Agar Anaerobic Plates.—A comparison of these two culture plates has been found to give a very satisfactory index of the relative numbers of *B. acidophilus* which may be present in the stool. The various types of the colon group and the streptococci develop on both media, but the bacillus acidophilus and, at times, the bacillus bifidus appear only on the anaerobic lactose plates. The bacillus acidophilus develops very feebly, if at all, on media without sugar, and altho it will grow aerobically a higher degree of development occurs under anaerobic conditions. Such anaerobic lactose plates, seeded from stools containing many bacilli of

11. Arch. f. Hyg., 1904, 49, p. 229.

the acidophilus type, exhibit a peculiar and characteristic grouping of the colonies. At suitable dilutions, the colonies are seen to be grouped in clusters. In the center, there is at least one large lenticular colony which may or may not have an associated bubble of gas. Clustered about these central colon bacillus colonies are smaller colonies, often in great numbers, which decrease in size with increasing distance from the central colony until, at the periphery, they become exceedingly minute. In the neighborhood of these colony groups, the medium is clouded. The smaller colonies are composed of the bacillus acidophilus or, at times, of the bacillus bifidus and it is evident that their development is initiated and favored by the acid and possibly other growth

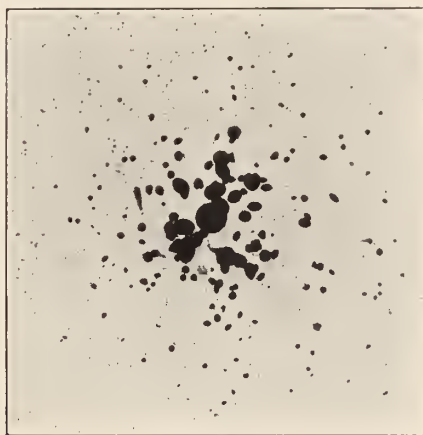


Fig. 2.—Detail of one of the clusters. X4.

products formed by the colon bacilli diffusing through the medium. These bacilli are evidently acidophilic as well as aciduric.¹² In some instances, the large central colonies were found to be composed of aciduric bacilli. In the culture plates containing several hundred colon bacilli the development of these acidophilic bacteria may be uniform throughout the plate. The difference in the count on the anaerobic lactose agar plates, exhibiting this peculiar grouping of the colonies and clouding of the medium, and that on the sugar-free agar plates or gelatin plates may be considered a fair index of the number of viable acidophilic bacilli in the stool. In the tables directly under the count

12. Kendall has suggested this name for the whole group of acid-tolerating bacteria, *Jour. Med. Research*, 1910, 22, p. 153.

for the anaerobic lactose agar plates, the type of the predominating microorganism is stated.

Acetic Acid Glucose Broth.—Additional information in regard to the acidophilic flora is gained from an inspection of the tubes containing three strengths of acetic acid, each of which has been seeded with 5 mg. of the feces. Aside from yeasts, the bacillus acidophilus was the only organism found in these stools capable of multiplying in this acid medium. While the lactose agar anaerobic plates reveal the number of *B. acidophilus* in the stool, the acid fluid media indicate especially the aciduric properties of these bacilli. Organisms which are capable of multiplying in a medium with an acidity of N/10, or even N/5, acetic acid have acquired marked acid resisting properties. An examination of stools from normal individuals, and also from those with a somewhat disturbed digestion, has shown that such highly aciduric bacilli are only rarely present in the intestinal tract. As a result, however, of a continued diet containing large amounts of carbohydrate associated with reduced amounts of protein and fat, such bacilli may be developed (Table 1). Their presence in the stool justifies the assumption that unusual amounts of lactic acid are being produced in the intestinal tract, especially in the region of the large intestine, which is the locality where these bacilli undergo their greatest development. It may also be assumed that carbohydrate is available in this locality for their metabolism.

Fermentation Tubes.—Herter and his associates have laid much stress on the value of fermentation tubes containing sugar broths as an aid to the bacterial analysis of the feces. Because of the transition from almost complete anaerobiosis to aerobiosis which such tubes offer, they believed that the bacterial development in the tubes represents closely the viable organisms originally present in the feces. Furthermore, certain bacteria, such as the bacillus bifidus and the bacillus aerogenes capsulatus, altho present in such small numbers in the stool as to be overlooked, may be brought to strong development in these tubes. They asserted also that the volume of gas produced in tubes seeded with the feces is of significance, being much reduced from the normal 25-30 percent in certain intestinal disorders. Too much stress, however, should not be laid upon the volume of gas, as MacNeal, Latzer, and Kerr encountered a great variation in the amount produced in the case of twelve supposedly normal individuals exam-

ined at frequent intervals for an extended period. Kendall¹³ has found that, under normal conditions of the digestive system, closely similar amounts of gas are formed in the dextrose, lactose, and saccharose tubes.

In the tables, the amounts of gas formed in twenty-four hours are expressed in percentages. As the length of the closed arm of the fermentation tubes varied considerably, the figures given have greater comparative value than those obtained by direct measurement in millimeters.

In these typhoid cases, there was no definite deviation from the normal average in the amount of gas produced. The stools of patients on the high calory diet in the earlier stages of the infection, and also patients on a milk diet (Table 5), showed, for the most part, a somewhat greater production of gas with dextrose than with the other two sugars, whereas in the later periods generally after a rather prolonged feeding with lactose, the maximum amount of gas was formed in lactose. As has been observed by others, stools containing an abnormal proportion of streptococci give rise to a much reduced amount of gas, especially in the saccharose tube. In the cases of John T. (Table 1) and Howard F. (Table 3), this was regularly the case as regards saccharose. On the other hand, with increasing numbers of the bacillus acidophilus in the stools, the productions of gas in the dextrose and lactose tubes remained about equal. The largest amounts of gas did not occur during periods when the patients were fed large amounts of carbohydrates, as might be expected, but were encountered when the amount of fat in the diet was increased, and especially when the proportions of fat and protein to the carbohydrate were raised (Table 1, Enrico P.; Table 2, Christian M.; Table 5, Joseph P. and Torello D.).

A much larger amount of gas was regularly formed in the lactose bile fermentation tube than in the lactose broth tube, which was due, in part, to the heavier seeding of the former. These bile tubes offered an enrichment medium for the typhoid bacillus, which was of doubtful value; they were chiefly of service in permitting the development of the bacillus aerogenes capsulatus and other spore-bearing anaerobes of a more or less putrefactive type. An examination of the sediments from these tubes threw some light on the question of the fermentative or putrefactive nature of the feces with which they were seeded. Accordingly, these sediments have been classified in the tables in

13. Jour. Biol. Chem., 1909-10, 6, p. 257.

reference to their probable potential activity. Thus, smears from sediments showing a majority of the organisms of the bacillus acidophilus type are designated fermentative; those in which the larger numbers of the bacteria are of the colon and streptococcic types are termed peptolytic; while smears in which there were many bacilli of the aerogenes capsulatus type, together with other spore-bearing bacilli are classified as putrefactive.

Another medium regularly employed in fermentation tubes was litmus milk. Stools, in which the bacillus acidophilus is predominant, generally form a rather soft clot with little or no gas; with many streptococci, the clot is firmer and there is little gas; with the ordinary stool of the adult the clot is solid and shrunken, whey is expressed from the clot and there is formed 20-50 percent of gas. A production of 100 percent gas generally, but not always, means the presence of large numbers of *B. welchii*. The principal object in using these milk fermentation tubes was to detect the presence of bacteria able to digest casein under anaerobic conditions. For this purpose, the tubes were kept under observation for a period of about ten days. This occurred only rarely and in a moderate degree.

Loeffler Serum Medium Slant and Gelatin Stab Culture.—These media were inoculated with the undiluted feces in order to gain an idea of the relative numbers of bacteria in the various stools capable of digesting proteins of this character. The aerobic liquefying bacteria are supposed to develop especially in the small intestine. Herter and Kendall⁵ determined, through feeding experiments, that the fecal flora of monkeys, placed on a high protein diet, caused a rapid liquefaction of these media, whereas when the monkeys were given a diet consisting largely of carbohydrate slight or no digestion occurred. With typhoid patients exhibiting a fermentative flora, there occurred slight or no digestion of serum medium, whereas the liquefaction of the gelatin medium was often marked.

Spore-Bearing Bacteria.—As has already been mentioned a suspension of the feces to the amount of 1 mg. per cubic centimeter was heated at 80 C. for fifteen minutes. One cubic centimeter of this heated suspension was plated in sugar-free agar and incubated aerobically at 37 C.; 1 c.c. in lactose agar and incubated aerobically at 37 C.; and in some instances, 1 c.c. was plated anaerobically in lactose agar to which 1 c.c. of defibrinated blood had been added and also

seeded in blood milk fermentation tubes, both being incubated at 37 C. The last two media favored especially the development of *B. welchii*. Comparative results indicated that about ten times as many spores of this type would develop in the blood lactose agar plates as in the same media without blood. When these spores were present in small numbers in the stools, under 40 per milligram, the characteristic growth reaction with disruption and partial digestion of the curd might be delayed for forty-eight to seventy-two hours.

MacNeal found in normal human stools an average per milligram of 65 spores developing aerobically and 1,790 anaerobically. In these stools from typhoid cases on the high calory diet, I have rarely found as many spores as this; in fact, in many instances, no colonies at all developed on the plates seeded with the heated suspension of the feces. Those which did appear on the anaerobic plates were almost invariably of the bacillus welchii type. Their occurrence in relation to the diet will be discussed in the following section.

Direct Smears from the Feces Stained with Gram's Method.—In the original tabulations of the bacteria observed in the gram-stained fecal smears, fourteen different types were enumerated. In compiling the tables, however, it has been necessary to condense this enumeration and to group the bacteria under a few general types, which may lay claim to some distinction in morphology or staining, and which reacted definitely to changes in diet. The gram-negative bacilli were almost entirely of the colon type. The gram-negative cocci were present in such small numbers, if at all, as to be negligible. No spirochetes were observed. Within the aciduric bacilli group have been placed nearly all of the gram-positive rods without spores seen in the fecal smears. A study of pure cultures of aciduric bacilli isolated from these stools has shown that they may vary markedly in morphology. The most frequently encountered type, taken for the bacillus acidophilus, was a rather long, slender bacillus, about 3-6 microns, with rounded ends and strongly gram-positive. This bacillus, however, often occurs as a gram-positive, rather short, plump rod, 1-3 microns, and with rounded ends. There are also gram-positive, long, thick rods which belong in the aciduric group, but which cannot, from their morphology alone, be distinguished from the non-spore-bearing representatives of the bacillus aerogenes capsulatus. Certain gram-positive bacilli of the length of the first type of the bacillus acidophilus mentioned, but rather thicker, often curved and irregularly

staining, and with a punctate or bifid end, were not infrequently encountered. These were regarded as representatives of the bacillus bifidus and placed in the aciduric group. Logan¹⁴ has recently directed attention to the marked polymorphism displayed by the various members of this acid-tolerant group.

The tables show a fairly close correspondence between the results obtained by the cultural procedures and the direct counts from the fecal smears. Of the two, however, the cultural tests give the more reliable information in regard to the actual bacterial conditions in the intestinal tract. This is due, in part, to the uncertainty of identification of the more important intestinal bacteria by morphology alone, and, in part, to the fact that an inspection of a gram-stained smear gives no information in regard to the viability of the bacteria. The number of viable bacteria in a stool in ratio to the total count has been estimated at anywhere from 1-100 (Klein) to 1-3,000 (MacNeal). Where there is a radical lack of correspondence in the tables between the cultural results and the direct count, it is probable that the discrepancy is due to an unusually large proportion of dead bacteria. This was especially apt to occur following marked changes in the diet.

In a number of cases, gram-stained smears from the sediments of the broth fermentation tubes after twenty-four hours' incubation were compared with direct counts from the stool. A comparison of sediments of the dextrose, lactose, and saccharose tubes, seeded from the same material, did not show any uniform difference in the character of the sediments, altho, in some instances, streptococci had multiplied more actively in the saccharose tube, and the bacillus bifidus appeared in larger numbers in the sediments from the dextrose and lactose tubes. The counts indicate, as Herter and Kendall¹⁵ have claimed, that the growths in these fermentation tubes represent, in a measure, the bacteria originally present in the feces. They are not present however in the same relative numbers. Streptococci and also the bacillus acidophilus, if present in any considerable numbers in the stool, always multiplied to such a degree as to far outnumber the colon bacillus types in the sediments. The most interesting type sometimes brought to active development was the bacillus bifidus. This occurred at times in tubes seeded with feces in the smears of which the bacillus bifidus was seen in very small numbers, or not at all.

14. Jour. Path. and Bacteriol., 1914, 18, p. 527.

15. Jour. Biol. Chem., 1908-9, 5, p. 283.

It may be mentioned that in two watery stools with much mucus from two typhoid patients with active diarrhea the bacillus bifidus, including many bifid forms, was observed in the fecal smears, in very large numbers, in one instance constituting 55 percent of the total bacteria, and in the other 29 percent. As this bifid bacillus has been encountered rarely in the stools of normal adults, its presence in such large numbers in these pathological adult stools is worthy of note.

DESCRIPTION OF THE TABLES

It was not feasible to attempt the detailed publication in a tabulated form of the findings in the total 103 stools examined. Accordingly, only a number of selected cases have been presented in such detail as space permitted. These, however, illustrate the salient features revealed in this work. In each of Tables 1-4 have been grouped two or more cases which illustrate the response of a certain type of fecal flora to various modifications of the high calory diet, while in Table 5 are given the results with a milk diet in two cases. In the following section, there is a discussion of the various types of flora encountered and their reactions to the different diets.

All of the counts from the plate cultures refer to the numbers of colonies appearing per milligram of the feces. The figures for the fermentation tubes indicate the percentage of gas formed. In indicating the degree of liquefaction of gelatin and Loeffler serum, the following symbols are used: \pm , slight; +, moderate; ++, marked; +++, rapid; +++++, very rapid. As regards the amount of growth in the acetic acid glucose broth tubes: 1 means slight growth; +, moderate; ++, heavy; +++, very heavy.

In Tables 1-4, the average daily amounts of proteins, fats, and carbohydrates given each patient are expressed in grams. Unless otherwise stated, the figures signify the average for the period since the last examination. In the first column, the diet for each patient since admission to the hospital is described.

The following brief case-reports include only such of the patients on the high calory diet as are incorporated in the tables.

John T.—Twenty-five years old, admitted November 19, 1912, on the eighth day of the disease. Onset five days ago with headache and vomiting. Since then the patient has had anorexia, malaise, and several nose bleeds. Looks fairly well nourished but weak and acutely ill.

On November 21, agglutination test positive and typhoid bacilli recovered from the blood; November 20, several rose spots appeared, small abdominal

hemorrhage in the evening; November 23, spleen palpable; November 25, abdomen slightly tympanitic, phlebitis in right thigh; November 26 to 29 and on December 1 patient had chills; December 4, general condition improved, patient less stuporous; December 31, temperature shot to 103, both femoral regions painful and swollen; January 1, temperature normal. Right leg remained swollen during convalescence. Patient gained rapidly in weight.

Enrico P.—Twenty-four years old, admitted December 4, 1912, on the fourth day of the disease. Two days ago had severe cough with blood-tinged expectoration. Appetite failed and patient suffered from headache. Looked fairly well nourished, but acutely ill; many large and small moist râles in lungs; spleen palpable.

December 5, agglutination test positive and typhoid bacilli recovered from the blood, patient apathetic, expectoration and headache continues, no rose spots; December 10, abdomen slightly distended, signs of bronchitis; December 14, spleen palpable 2 cm. below costal margin, general condition good, nutrition good; January 3, stools continue green and contain mucus, diet almost exclusively carbohydrate had not changed character of the stools, patient put on fat diet for three days, stools changed markedly, became light brown and formed, takes fat diet well; January 15, patient seems to have intolerance for lactose, whenever given 50 to 60 gm. shows distention, passes large amounts of gas and suffers considerable abdominal pain. Convalescence rapid. Patient kept for examination of feces.

Anthony G.—Eight years old, admitted August 19, 1913, on the eleventh day of the disease. About two weeks ago began to be apathetic, lost appetite, and suffered from headaches; in bed since August 15. Looked fairly well nourished and well developed; spleen not palpable; several rose spots; agglutination doubtful.

On August 23, severe abdominal pains, no sign of perforation, abdomen much distended; August 25, many rose spots, spleen palpable. No more abdominal distention. Convalescence rapid.

Christian M.—Thirty-one years old, admitted September 8, 1913, on the eleventh day of the disease. About two weeks ago began to have headaches and malaise, four days ago stopped work, next day developed diarrhea. Very well developed; apathetic.

On September 8, agglutination was positive and typhoid bacilli recovered from the blood, spleen not palpable, rose spots appeared in crops; September 19, pain and tenderness in right lumbar region lasting several days; October 4 to 15, typhoid bacilli in urine and feces, disappearing later.

Thomas B.—Sixty years old, admitted October 2, 1913, on the fifteenth day of the disease. For two weeks has suffered from malaise and anorexia; has been feverish. A large, well-developed man, looks acutely ill, apathetic.

On October 21, many rose spots, agglutination positive, blood culture positive for typhoid bacilli.

October 13, rose spots on abdomen and back, spleen not palpable; October 27, uninterrupted convalescence; November 10 to 26, serofibrinous pleurisy, fluid base of left lung.

Morris S.—Twenty-one years old, admitted August 17, 1913, on the seventh day of the disease. Seven days ago began to have pains in abdomen and back and since then loss of appetite and constipation. He has a short, small frame, well nourished; distinct pyorrhea alveolaris; spleen palpable.

On October 18, agglutination negative, blood culture positive for typhoid bacilli; October 22, complains of sore throat; October 23, condition good, slightly apathetic, spleen palpable 4 cm. below costal margin, abdomen slightly distended;

October 24, agglutination positive; October 27, abdomen which was distended on high carbohydrate and very low fat diet became soft when the amount of fat was increased; October 30, a little irrational, color grayish, pulse soft; November 4, general condition improved; November 17, temperature rising, patient feels well, does not look sick; November 18, sharp pains right side of abdomen lasting a few hours; November 24, patient not toxic in this relapse; December 3, feels well, patient had frothy stools on high carbohydrate, but when amount of fat increased became normal; December 17 to 25, second relapse, patient not at all sick, spleen not palpable, no rose spots. Throughout the long febrile period the patient's state of nourishment remained very good and convalescence was rapid.

Howard F.—Twelve years old, admitted November 4, 1913, on the fourth day of the disease. On October 26 severe headaches and malaise; October 29, developed chills and took to bed. His uncle, Charles F., living in the same house developed typhoid at the same time. Tall, well-developed boy; teeth good; good state of nutrition; spleen not palpable; several rose spots.

On November 8, condition good, rational but apathetic; November 12, signs of bronchitis; November 14, blood culture positive for typhoid bacilli; November 17, takes food poorly, much emaciated, somewhat toxic; December 10, passed two ascarides. Convalescence rather slow, heart somewhat enlarged, action rapid on exertion.

Charles F.—Twenty-four years old, admitted November 4, 1913, on the eighth day of the disease. About a week ago lost appetite and since then has suffered from headaches and malaise. Medium frame, well nourished, spleen palpable, several rose spots.

On November 5, agglutination positive; November 6, blood culture positive for typhoid bacilli; November 8, rational, slightly toxic, several small intestinal hemorrhages; November 19, intestinal hemorrhage of about 250 c.c., patient much prostrated, takes food badly, many rose spots in crops; November 14 to 17, developed severe follicular tonsillitis, became very toxic and apathetic, pulse of poor quality; December 3, condition much improved. Convalescence fairly rapid.

Thomas F. Thirty-four years old, admitted August 22, 1913, on the fourteenth day of the disease. About two weeks ago began to suffer from headaches and malaise. Well developed and well nourished; rough systolic murmur at base of heart; spleen not palpable.

On August 27, many rose spots; August 29, very apathetic; September 2, blood culture positive for typhoid bacillus, small abscesses on buttocks, has had much abdominal distention at times, irrational; September 5, abscesses incised; September 8, emaciation has been very rapid; September 12, showed much improvement. Convalescence rather slow.

Emil C.—Twenty-three years old, admitted August 23, 1913, on the fifth day of the disease. Five days ago suffered from fever, chills and headache; four days ago colicky pains in stomach, appetite good. Well nourished, does not look acutely ill; spleen palpable; doubtful rose spots.

On August 26, agglutination positive; August 29, blood culture positive for typhoid bacilli; September 1, more rose spots, severe headache; September 5, abdominal distention moderate; September 19 to 21, complains of headache; September 24, began to relapse; September 26, temperature rose to 104 F., spleen palpable 6 cm. below costal margin, several rose spots. During convalescence, October 18 to 30, temperature elevated, cause unknown.

TABLE 1

EFFECT OF HIGH CALORY DIET CONTAINING MUCH CARBOHYDRATE ON THE FECAL FLORA OF TWO TYPHOID PATIENTS WITH INITIAL FACULTATIVE TYPES OF FLORA
 John T., Age 25, Admitted Nov. 19, 1912, on Eighth Day of Disease. Convalescent, Jan. 1, 1913. Moderately Severe Typhoid.

Date	Nov. 22	Nov. 25	Nov. 29	Dec. 6	Dec. 12	Dec. 19	Dec. 27	Jan. 15
Character of stool.....	Semi-fluid (much blood)	Fluid (no blood)	Semi-formed	Semi-formed	Semi-formed	Formed	Formed	Formed
Endo plate.....	++ B. coli B. typhosus Streptococcus	++++ Streptococcus B. coli	++++++ Streptococcus B. coli	++ B. coli Streptococcus	++ B. coli Streptococcus	+	+++++	++ B. coli Streptococcus B. aerogenes
Typhoid bacillus.....	+	—	—	—	—	—	—	—
Lactose agar plate, anaerobic, 37 C.	140,000 B. coli	750,000 Streptococci	3,000,000 Streptococci	1,750,000 B. acidophilus	500,000 B. acidophilus	240,000 B. acidophilus	1,100,000 B. acidophilus	40,000 B. acidophilus
Gelatin plate, aerobic, 21 C.	110,000	1,000,000 Streptococcus	450,000	8,000	3,500	1,500	500,000	39,000
Sugar {Dextrose	20	2	5	25	35	32	32	25
broth fer- {Lactose	30	5	10	20	20	35	35	40
mentation {Saccharose	10	0	2	10	15	28	28	15
Acetic acid glucose broth N/20.	—	+	1	++	++++	++	++	+
Acetic acid glucose broth N/10.	—	—	—	++	++	++	++	1
Acetic acid glucose broth N/5.	—	—	—	+	++	+	+	—
[Total gram-positive bacteria.	71.7	98.0	75.7	100.0	94.0	52.0	84.6	75.3
Sinear {Total gram-negative bacilli...	23.3	2.0	24.3	0	6.0	48.0	15.4	24.7
from {Total aciduric bacilli.....	28.3	30.6	19.2	97.4	81.9	38.3	69.2	71.9
stool {Total streptococci	5.0	33.3	37.6	2.6	4.0	7.6	3.6	.6
[Total spores	0.6	.0	1.6	.0	.0	.0	2.3	.0
Diet {Protein	Milk diet	38.3	77.0	120.0	121.5	120.5	120.5	99.5
in {Fat	250-750 c.c.	73.3	103.0	146.6	178.3	198.3	205.0	165.0
grams {Carbohydrate	243.6	287.5	361.6	408.8	442.5	451.2	409.5
Temperature	104	103.5	106	103.2	101.3	100	99.5	98.4

TABLE 1—(Continued)
 Enrico P., Age 24, Admitted Dec. 4, 1914, on Fourth Day of Disease. Convalescent, Feb. 11, 1914. Moderately Severe Typhoid.

Date	Dec. 7	Dec. 14	Dec. 23	Jan. 10	Feb. 6	Feb. 11	Feb. 21	Feb. 26
Character of stool.....	Semi-formed	Pultaceous	Semi-formed	Formed	Semi-formed	Semi-formed	Formed (moist)	Semi-formed
Endo plate.....	++ B. coli	++++ B. coli	++ B. coli B. aerogenes	++++ Streptococcus B. coli B. aerogenes	++ B. coli B. aerogenes	1 Streptococcus B. coli	+++ B. coli	+ B. coli B. aerogenes
Typhoid bacillus.....	—	—	—	—	—	—	—	—
Lactose agar plate, anaerobic, 37 C.	260,000 B. coli	4,200,000 Streptococci	450,000 B. acidophilus	2,000,000 B. acidophilus	1,000,000 B. acidophilus	200,000 B. acidophilus	500,000 B. coli	3,300 B. coli
Gelatin plate, aerobic, 21 C.	520,000	7,600,000	600	31,000	5,000	900	370,000	2,400
Sugar (Dextrose	40	—	25	28	25	30	45	60
broth fer- Lactose	35	30	28	28	30	28	55	52
mentation Saccharose	25	10	25	25	33	25	40	48
Acetic acid glucose broth N/20.....	+	++	++	++	+	+	+	1
Acetic acid glucose broth N/10.....	—	+	+	+	1	+	+	—
Acetic acid glucose broth N/5.....	—	—	—	1	—	—	—	—
(Total gram-positive bacteria from Total aciduric bacilli stool) Total streptococci Total spores	58.9 41.1 27.9 6.6 .3	46.6 53.4 38.3 1.0 .0	100.0 .0 99.2 1.6 .0	97.0 3.0 95.3 2.0 .0	84.3 15.7 57.6 2.6 .0	86.7 13.7 84.2 2.3 .0	5.6 94.4 4.0 .3 1.3	42.5 57.5 34.3 2.0 2.6
Sugar (Total gram-negative bacteria from Total aciduric bacilli stool) Total streptococci Total spores	58.9 41.1 27.9 6.6 .3	46.6 53.4 38.3 1.0 .0	100.0 .0 99.2 1.6 .0	97.0 3.0 95.3 2.0 .0	84.3 15.7 57.6 2.6 .0	86.7 13.7 84.2 2.3 .0	5.6 94.4 4.0 .3 1.3	42.5 57.5 34.3 2.0 2.6
Diet (Protein	Milk diet for 4 days	73.0 94.8	79.2 124.2	65.1* 171.7	110.4† 284.1	144.5 153.0	131.8 158.0	111.1 149.6
in Fat	grams	240.3	371.6	259.0	284.1	328.4	146.6	123.3
Carbohydrate	103.5	104	102	99.5	98.4	98.0	98.4	98.4
Temperature								

* Average past 7 days.

† Average past 6 days.

TABLE 2

EFFECT OF HIGH CALORY DIET ON INTESTINAL BACTERIA OF TYPHOID PATIENTS WITH AN INITIAL FERMENTATIVE TYPE OF FLORA

Anthony G., Age 18, Admitted Aug. 19, 1913, on Eleventh Day of Disease. Convalescent, Sept. 8, 1913. Mild Typhoid.

Date	Aug. 22	Aug. 26	Sept. 2	Sept. 8
Character of stool.....	Pultaceous	Pultaceous	Pultaceous	Semi-formed
Endo plate.....	+++ Streptococcus B. coli B. aerogenes	++ B. coli B. aerogenes Streptococcus B. typhosus	++++ B. coli B. aerogenes Streptococcus	++ B. coli Streptococcus B. aerogenes
Typhoid bacillus.....	—	+	—	—
Sugar-free agar plate, aerobic, 37 C.	225,000	100,000	850,000	100,000
Lactose agar plate, anaerobic, 37 C.	1,450,000 B. acidophilus	1,400,000 B. acidophilus	1,300,000 B. acidophilus	1,350,000 B. acidophilus
Spores, aerobic, 37 C.	1	0	1	0
Spores, anaerobic, 37 C.	0	0	0	0
Lactose bile fer- (Gas mentation tube) (Sediment)	40 Peptolytic	30 Fermentative	50 Fermentative	45 Fermentative
Gelatin stab, 21 C.	+	+	+	—
Loeffler serum slant, 37 C.	++	±	±	±
Sugar { Dextrose	30	25	38	40
broth fer- { Lactose	23	28	35	45
mentation { Saccharose	28	18	25	15
Acetic acid glucose broth N/20.....	++	++	++	++
Acetic acid glucose broth N/10.....	+	+	+	+
Acetic acid glucose broth N/5.....	—	—	—	—
Smear { Total gram-positive bacteria..	66.0	100.0	92.0
from { Total gram-negative bacilli...	34.0	0.0	8.0
stool { Total aciduric bacilli.....	62.2	98.6	89.9
{ Total streptococci	0.3	0.6	1.0
{ Total spores	0.0	0.0	0.0
Diet { Protein	Milk diet	44.2	96.8*	108.0
in { Fat	since	57.6	177.3	212.3
grams { Carbohydrate	Aug. 19	149.9	359.5	370.3
Temperature	103.2	101.6	99.9	98.6

* Average for 2 days.

TABLE 2.—(Continued)

Christian M., Age 31, Admitted Sept. 8, 1913, on Eleventh Day of Disease. Convalescent, Sept. 23, 1913. Mild Typhoid.

Date	Sept. 17	Sept. 22	Sept. 27	Sept. 30
Character of stool.....	Pultaceous	Formed	Formed (rather dry)	Pultaceous
Endo plate.....	++++ B. coli Streptococcus B. aerogenes	++++ B. coli Streptococcus B. aerogenes	+++ Streptococcus B. coli Staphylococcus	++++++ Streptococcus B. coli B. paracoli
Typhoid bacillus.....	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	520 000	325,000	56,000	1,100,000
Lactose agar plate, anaerobic, 37 C.	2,730,000 B. acidophilus	3,450,000 B. acidophilus	50,000 B. coli	4,100,000 B. acidophilus
Spores, aerobic, 37 C.	0	0	7	0
Spores, anaerobic, 37 C.	0	0	0	0
Lactose bile fer-(Gas	33	65	75	48
mentation tube(Sediment	Fermentative	Fermentative	Peptolytic	Fermentative
Gelatin stab, 21 C.	+	++	+	+++
Loeffler serum slant, 37 C.	±	±	++	++
Sugar {Dextrose	18	18	35	12
broth fer- {Lactose	20	33	45	18
mentation {Saccharose	20	10	18	10
Acetic acid glucose broth N/20.....	++	+++	1	+++
Acetic acid glucose broth N/10.....	+	++	—	++
Acetic acid glucose broth N/5.....	—	1	—	—
Smear {Total gram-positive bacteria.	52.0	55.3	86.6	36.3
from {Total gram-negative bacilli...	48.0	44.7	14.4	63.6
stool {Total aciduric bacilli.....	48.6	45.9	63.2	30.9
{Total streptococci	1.3	1.3	4.0	2.6
{Total spores	0.0	0.0	0.0	0.0
Diet {Protein	80.5*	94.1	96.8	111.8
in {Fat	122.1	74.2	206.5	155.9
grams {Carbohydrate	349.6	483.4	209.8	457.6
Temperature	101.8	99.4	99.0	99.5

* Average for 3 days.

TABLE 2.—(Continued)

Morris S., Age 25, Admitted Oct. 17, 1913, Seventh Day of Disease. Relapsed Nov. 15-Dec. 5, 1913; relapsed Dec. 17-Dec. 25, 1913. Severe Typhoid.

Date	Oct. 24	Oct. 28	Nov. 6	Nov. 11	Nov. 24
Character of stool.....	Fluid	Pultaceous	Semi-formed	Pultaceous (frothy)	Fluid
Endo plate.....	+	++	++++	++	++
	B. coli Streptococcus	Streptococcus B. coli B. paracoli	B. coli	B. coli	B. aerogenes B. coli Streptococcus
Typhoid bacillus.....	—	+	+	—	—
Sugar-free agar plate, aerobic, 37 C.	16,000	100,000	520,000	48,000	250,000
Lactose agar plate, anaerobic, 37 C.	1,200,000 B. acidophilus	1,800,000 B. acidophilus	6,800,000 B. acidophilus	1,900,000 B. acidophilus	1,170,000 B. acidophilus
Spores, aerobic, 37 C.	0	5	0	0	1
Spores, anaerobic, 37 C.	0	0	0	0	1
Lactose bile fer-(Gas	66	75	75	75	72
mentation tube/Sediment	Fermentative	Fermentative	Fermentative	Fermentative	Fermentative
Gelatin stab, 21 C.	±	+	±	++	±
Loeffler serum slant, 37 C.	+++	++	++	+	±
Sugar {Dextrose	20	65	20	16	28
broth fer- {Lactose	18	32	20	22	18
mentation {Saccharose	2	10	5	28	8
Acetic acid glucose broth N/20.....	+++	+++	+++	++	++++
Acetic acid glucose broth N/10.....	+++	++	+++	+	+++
Acetic acid glucose broth N/5.....	++	+	+	—	1
Smear {Total gram-positive bacteria.....	74.3	85.7	58.4	88.0	85.7
from {Total gram-negative bacilli.....	25.7	14.3	41.6	12.0	14.3
stool {Total aciduric bacilli.....	64.1	75.6	52.2	85.6	84.2
{Total streptococci	2.6	6.0	1.6	0.0	0.6
{Total spores	0.0	0.0	0.0	0.0	0.0
	(Many yeasts)		(Many yeasts)		
Diet {Protein	Oct. 21-24.	109.8	95.1	95.4	59.9
in {Fat	2,000-3,000 calo-	80.9	129.9	143.3	107.9
grams {Carbohydrate	ries with high carbohydrate	324.9	295.9	319.1	203.1
Temperature	103	103.5	102	99.5	104

* See Page 34.

EFFECT OF MODERATELY HIGH CALORY DIET ON THE FECAL FLORA OF TWO SEVERE TYPHOID PATIENTS WITH AN INITIAL FACULTATIVE TYPE OF FLORA
Howard F., Age 12 Years, Admitted Nov. 4, 1913, on Fourth Day of Disease. Convalescent, Nov. 28, 1913. Severe Typhoid.

TABLE 3

Date	Nov. 7	Nov. 11	Nov. 17	Nov. 21	Nov. 28	Dec. 10
Character of stool.....	Formed (moist)	Formed (moist)	Pultaceous	Semi-formed	Formed (moist)	Formed (moist)
Endo plate.....	+++ B. coli Streptococcus	+++ B. coli B. aerogenes Streptococcus	+++ Streptococcus B. coli	+++ B. coli Streptococcus	+++++ Streptococcus B. coli	+++ B. coli
Typhoid bacillus.....	—	+	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	175,000	96,000	300,000	545,000	1,130,000	480,000
Lactose agar plate, anaerobic, 37 C.	143,000 B. coli	170,000 B. coli	550,000 B. acidophilus	885,000 B. acidophilus	1,350,000 Streptococci	1,150,000 B. acidophilus
Spores (Aerobic, 37 C. Anaerobic lactose agar, 37 C. Anaerobic blood lactose agar, 37 C.	0 0	0 2	0 0	0 0 38	5 450 10,000	3 1 34
Gelatin stab 21 C.	—	±	±	±	+	+
Loeffler serum slant, 37 C.	++	+++	+++	+++	+	+
Sugar (Dextrose broth fer-Lactose mentation) Succharose	35 42 5	50 30 18	40 30 10	25 38 10	30 40 5	20 18 2
Acetic acid glucose broth N/20..... Acetic acid glucose broth N/10..... Acetic acid glucose broth N/5.....	+++ — —	++ + —	+++ +++ —	+++ ++ —	+++ ++ —	+++ ++ —
Total gram-positive bacteria Smear Total gram-negative bacilli from Total aciduric bacilli stool Total streptococci (Total spores	57.3 42.7 52.5 4.3 0.0	60.0 40.0 55.3 4.3 0.0	93.6 6.4 85.6 8.0 0.0	93.3 6.3 92.2 3.6 0.0	13.3 86.7 10.6 2.3 0.0	54.0 46.0 52.2 2.6 0.0
(Many yeasts)						
Diet (Protein in Fat grams Carbohydrate	80.9 80.8 42.1	46.6 79.0 91.8	36.7 46.8 101.4	43.3 71.4 97.8	68.2 119.5 132.4	99.5 120.4 352.0
Temperature	102.2	104	102.6	103.2	98.4	99

TABLE 3—(Continued)
Charles F., Age 24 Years, Admitted Nov. 4, 1913, on Eighth Day of Disease. Convalescent, Dec. 2, 1913. Severe Typhoid.

Date	Nov. 7	Nov. 10	Nov. 17	Nov. 21	Nov. 28	Dec. 10
Character of stool.....	Formed (dry)	Pultaceous	Semi-formed	Fluid	Formed (rather dry)	Semi-formed
Endo plate.....	B. coli +	B. coli + B. paracoli	Streptococcus B. coli +++++	Streptococcus B. paracoli, B. coli +++++	Streptococcus B. coli ++	Streptococcus B. coli ++
Typhoid bacillus.....	—	—	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	235,000	60,000	235,000	520,000	230,000	210,000
Lactose agar plate, anaerobic, 37 C.	175,000 B. coli	85,000 B. coli	340,000 B. coli	91,000 B. coli	365,000 B. acidophilus	365,000 Streptococci
(Aerobic, 37 C.	0	5	1	11	46
Spores (Anaerobic lactose agar, 37 C.	0	0	5	0
(Anaerobic blood lactose agar, 37 C.	40	2	4
Gelatin stab 21 C.	+	++	+	—	+	++
Loeffler serum slant, 37 C.	—	+	+++	±	+	+
Sugar (Dextrose	40	17	25	38	20	25
broth fer- Lactose	40	23	15	28	35	33
mentation) Saccharose	15	5	8	15	20	37
Acetic acid glucose broth N/20.	++	+	+	++	+++	+++
Acetic acid glucose broth N/10.	1	1	—	1	—	1
Acetic acid glucose broth N/5.	—	—	—	—	—	—
Total gram-positive bacteria	41.0	61.0	78.6	68.0	41.0	57.0
Total gram-negative bacilli	59.0	33.0	21.4	32.0	59.0	43.0
Total aciduric bacilli	33.6	53.6	68.6	64.6	32.2	52.2
Total streptococci	5.6	5.6	5.3	5.0	3.0	2.3
Total spores	0.0	0.0	0.0	0.0	0.0	0.0
(Many yeasts)						
Milk and whites of eggs	62.4	62.4	46.3	48.9	74.4	85.2
Diet { Protein			86.3	72.3	129.8	125.2
in { Fat			181.4	105.4	132.9	322.3
grams { Carbohydrate						
Temperature	104	103	105	104.6	103.4	99

TABLE 4

TYPHOID CASES WITH AN INITIAL PUTREFACTIVE FLORA

Thomas F., Age 34, Admitted Aug. 22, 1913, on Fourth Day of Disease. Convalescent, Oct. 2, 1913.
Severe Typhoid.

Date	Aug. 26	Aug. 29	Sept. 4	Sept. 13	Sept. 19
Character of stool.....	Formed (moist)	Pultaceous	Semi-fluid	Pultaceous	Pultaceous
Endo plate.....	++ B. coli Streptococcus B. pyocyaneus	+++ B. coli Streptococcus B. aerogenes	+++ B. coli Streptococcus B. aerogenes	+++ B. coli Streptococcus B. typhosus	++ B. typhosus B. alkaligenes B. coli
Typhoid bacillus.....	—	—	—	+	+
Sugar-free agar plate, aerobic, 37 C.	160,000	730,000	290,000	700,000	130,000
Lactose agar plate, anaerobic, 37 C.	180,000 B. coli	1,450,000 Streptococci	215,000 B. coli	900,000 B. coli	162,000 B. acidophilus
Spores, aerobic, 37 C.	5	0	625	0	2
Spores, anaerobic, 37 C.	560	0	0	0	0
Lactose bile fer-(Gas mentation tube)(Sediment)	60 Putrefactive	18	42 Peptolytic	35 Peptolytic	50 Peptolytic
Gelatin stab, 21 C.	++++	±	+++	±	++
Loeffler serum medium, 37 C.	++++	++	+++	+++	++++
Sugar {Dextrose	25	35	20	30	48
broth fer- {Lactose	30	25	35	28	30
mentation {Saccharose	20	12	15	15	12
Acetic acid glucose broth N/20.....	—	+	+	+	+
Acetic acid glucose broth N/10.....	—	1	—	—	+
Acetic acid glucose broth N/5.....	—	—	—	—	—
Total gram-positive bacteria	16.3	69.3	49.3	59.3	27.6
Total gram-negative bacilli	83.7	30.7	50.7	40.7	73.4
Total aciduric bacilli	14.2	29.6	42.3	63.6	25.2
Total streptococci	1.3	7.6	2.3	2.6	2.6
Total spores	0.0	2.3	0.0	0.0	0.0
Diet {Protein	Milk	48.4	50.1	84.4	94.8
in {Fat	since	78.6	71.6	159.2	173.1
grams {Carbohydrate	Aug. 22	135.6	144.7	260.6	480.6
Temperature	105	104.5	103	100	100.5

TABLE 4.—(Continued)

James C., Age 52, Admitted Aug. 24, 1913, on Seventeenth Day of Disease. Died Aug. 29, 1913. Emil C., Age 23, Admitted Aug. 23, 1913, on Fifth Day of Disease. Convalescent, Oct. 17, 1913. Severe Typhoid.

Date	James C. Aug. 25	Sept. 6	Sept. 16	Emil C. Sept. 22	Oct. 6
Character of stool.....	Semi-fluid	Pultaceous	Formed (moist)	Pultaceous	Formed (rather dry)
Endo plate.....	++ B. coli B. typhosus Streptococcus	+++ B. coli Streptococcus B. aerogenes	++ B. coli B. aerogenes Streptococcus	++++ B. coli Streptococcus B. aerogenes	++++ B. coli
Typhoid bacillus.....	+	—	—	—	+*
Sugar-free agar plate, aerobic, 37 C.	52,000	520,000	145,000	730,000	845,000
Lactose agar plate, anaerobic, 37 C.	53,000 B. coli	580,000 B. coli	160,000 B. coli	520,000 B. coli	660,000 B. coli
Spores, aerobic, 37 C.	0	0	280	20	33
Spores, anaerobic, 37 C.	85	2	20	0	35
Lactose bile fer-(Gas mentation tube)(Sediment	65 Peptolytic	60 Putrefactive	38 Putrefactive	70 Peptolytic	
Gelatin stab, 21 C.	+++	±	±	±	±
Loeffler serum medium, 37 C.	++	±	+	+++	++
Sugar {Dextrose broth fer- {Lactose mentation {Saccharose	40 35 25	40 20 15	20 25 25	30 60 38	40 40 10
Acetic acid glucose broth N/20.....	—	+	+	++	++
Acetic acid glucose broth N/10.....	—	—	—	—	—
Acetic acid glucose broth N/5.....	—	—	—	—	—
Total gram-positive bacteria	48.0	42.6	50.0	17.3	26.0
Total gram-negative bacilli	52.0	57.4	50.0	82.6	74.0
Total aciduric bacilli	10.6	30.6	41.9	17.3	20.9
Total streptococci	2.1	2.1	1.3	0.0	5.0
Total spores	0.0	0.0	0.0	0.0	0.0
Diet {Protein in {Fat grams {Carbohydrate	Milk	Milk (largely)	92.6† 210.2 161.6	93.9† 73.7 483.0	86.5 147.4 300.2
Temperature	104.5	104.5	102	100	103

† Average past 5 days.

* See page 34.

Joseph P., Adult, Admitted Nov. 1, 1913, to New York Hospital, in Third Week of Disease. Convalescent, Nov. 11, 1913. Mild Typhoid.
 Torello D., Adult, Admitted Nov. 1, 1913, to New York Hospital, in Fourth Week of Disease. Convalescent, Nov. 12, 1913. Mild Typhoid.

Date	Nov. 4	Nov. 8	Nov. 12	Nov. 4	Nov. 8	Nov. 12	Nov. 15
Character of stool.....	Formed (moist)	Pultaceous	Formed (moist)	Formed (rather dry)	Formed (very dry)	Formed (rather dry)	Semi-formed (moist)
Endo plate.....	+++ Streptococcus B. coli	++++ Streptococcus B. coli B. pyocyaneus	++++ B. coli Streptococcus B. typhosus	+++ B. coli	++++ Streptococcus B. paracoli B. coli	++++ Streptococcus B. paracoli B. coli	+++ B. coli Streptococcus B. paracoli
Typhoid bacillus.....	—	—	+	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	78,000	130,000	1,300,000	535,000	125,000	35,000	1,500,000
Lactose agar plate, anaerobic, 37 C.	910,000 B. acidophilus	475,000 B. acidophilus	1,300,000 B. coli	535,000 B. coli	90,000 B. coli	57,000 B. coli	1,200,000 Streptococcus
Gelatin plate, aerobic, 21 C.	100,000	105,000	1,250,000	795,000	150,000	11,000	1,400,000
Spores, aerobic, 37 C.	0	1	2	0	0	1	
Spores, anaerobic, 37 C.	7	0	0	0	0	0	
Lactose bile fer-(Gas mentation tube)(Sediment	50 Fermentative	20 Fermentative	35 Fermentative	85 Putrefactive	26 Peptolytic	62 Peptolytic	70 Peptolytic
Gelatin stab 21 C.	+	+	±	±	—	±	+
Loeffler serum slant, 37 C.	++	—	±	—	—	±	±
Sugar [Dextrose broth fer-(Lactose mentation)(Saccharose	30 18 5	45 20 12	62 45 42	50 38 18	20 17 8	40 30 27	50 35 15
Milk fer-(Gas mentation)(Peptonization	10 —	5 —	10 —	40 +	50 +	10 —	20 —
Acetic acid glucose broth N/20.....	++++	—	++	+	++	—	—
Acetic acid glucose broth N/10.....	++++	—	—	—	1	—	—
Acetic acid glucose broth N/5.....	1	—	—	—	—	—	—
[Total gram-positive bacteria Smear Total gram-negative bacilli from Total aciduric bacilli stool Total streptococci Total spores	99.3 0.7 92.8 6.3 0.0	88.6 11.4 79.8 3.3 0.0	81.0 19.0 76.2 1.3 0.0	41.2 58.7 33.6 3.0 0.0	47.0 53.0 61.2 1.3 0.0	42.6 57.4 37.2 3.6 0.0	48.2 51.7 44.6 3.6 0.3
Diet [Protein in Fat grams [Carbohydrate	History of milk diet with broth for 2 weeks	Daily average of 48 oz. of milk	Daily average of 44.5 oz. of milk	Milk diet since Nov. 1	Daily average of 54 oz. of milk	Daily average of 53 oz. of milk	Daily average of 56 oz. of milk
	102.8	100	98.4	103	103	95	99

GENERAL OBSERVATIONS

Aside from general observations in regard to the various types of intestinal flora which one may find associated with infection by the typhoid bacillus, several problems of a more special nature have been considered during the course of this investigation: How readily and to what degree can a typhoidal fecal flora be changed in character in response to variations in the constitution of the diet? Do patients with a particular type of fecal flora tolerate with special facility the high calory diet containing large amounts of carbohydrate? What is the effect of liberal amounts of carbohydrate on the intestinal bacteria? What effect has a diet containing much fat on the fecal flora? May an initial unfavorable fecal flora be changed to a favorable type through the use of an appropriate diet? Do patients with an initial favorable type of fecal flora exhibit, on the average, an enhanced resistance to the typical course of typhoid fever?

In respect to some of these points, the evidence has been definite, whereas for others the amount of material has been sufficient to offer no more than suggestions which will be followed out as far as possible in subsequent work.

Before entering upon a discussion of these questions, it is well to review briefly the nature of the intestinal bacterial conditions, as revealed by an examination of the stools, which conditions may be encountered in man after the period of early adolescence. One may find a flora of marked fermentative propensities. In such cases there is a persistence, in a somewhat modified form, of the flora frequently encountered in normal childhood. Bacteria of the bacillus acidophilus type are present in large numbers and may even be the dominant organisms encountered in an examination of the stools. These bacilli utilize little protein in their metabolism, but split many carbohydrates actively with the formation of much lactic acid and without gas production. Associated with these aciduric bacteria are bacilli of the colon group and moderate numbers of streptococci. There are few or no, spores to be seen in gram-stained smears, in which the majority of the organisms are positive to the stain, and few, or no, putrefactive bacteria are revealed by cultural tests. Strong growths occur in the acetic acid glucose broth tubes, while the liquefying action on gelatin is generally moderate, and on Loeffler serum medium is slight. The best single indicator of such a flora is the appearance of the anaerobic lactose agar plates of the peculiar clusters of colonies with a clouding of the medium, which has been described in detail in the preceding section. The persistence of such a purely fermentative flora is probably comparatively rare in adults on the ordinary mixed diet, but, on the other hand, adult individuals, in whom such a flora may be established through a diet for a brief period of large amounts of carbohydrate with reduced proportions of fat and protein, are not infrequently encountered. Examples of such a fermentative flora are detailed in Table 2.

At the other extreme, we find individuals who harbor an obligate putrefactive intestinal flora. In such a flora, the aciduric types are absent, the colon

bacilli are reduced in numbers, and the dominant organisms are a variety of anaerobic bacteria, many of which are spore bearing and all of which are either proteolytic or peptolytic. Streptococci and single cocci may be present in large numbers. As contrasted with a fermentative type, a putrefactive flora is notable for the markedly greater variety of bacterial species. In a cultural examination of such stools, no growth occurs in the acid broth tubes, gelatin and solidified serum are generally rapidly liquefied, and many colonies appear on anaerobic plates seeded from a suitable dilution of the heated emulsion of the feces. Such a flora, as Distaso¹⁶ has pointed out, is commonly found among persons subject to chronic constipation associated with intestinal stasis.

Between these two extremes are all gradations of which we may designate the central type as "facultative." In the typical facultative flora, the dominant organisms are bacilli of the colon type and the majority of bacteria in a direct smear from the feces are negative to the gram stain. The aciduric bacteria are present, but in small numbers, and the same is true of spore-bearing bacilli. Liquefying types and coccal forms may be fairly well represented. Such a flora is called "facultative" because the dominant bacteria may act either in a fermentative or a putrefactive manner in response to the conditions in the intestinal tract and the nature of the pabulum offered them. As has been demonstrated by Kendall and his co-workers,¹⁷ bacilli of the colon and proteus groups, in the presence of a suitable carbohydrate, attack the sugar in preference to the protein that may be present in the medium, but, in the absence of carbohydrate, members of these and other groups become peptolytic and may give rise to indol and other supposedly toxic bodies. The intestinal bacterial conditions in the majority of the typhoid cases investigated soon after entrance into the hospital were of this facultative type, in some instances verging strongly toward the putrefactive type (Table 4). In fact, the great majority of adults in ordinarily good health will be found to harbor such floras.

These rather well-known facts in regard to the various types of intestinal flora have been reviewed because they have an important bearing on the nature of the changes in the numbers and types of the bacteria which followed the use of various diets.

All of these typhoid patients, when placed on the high calory diet, were given at first comparatively large amounts of carbohydrate often in considerable measure in the form of lactose, together with much smaller amounts of fat and protein. As has been observed, it might be anticipated that this preponderance of carbohydrate in the diet would exert a sparing action on the bacterial protein metabolism and would tend to convert the intestinal flora to a purely fermentative type. The change was well marked in certain cases and less so in others; the degree of substitution depended largely on the type of flora which was present at the initiation of the attack. From time to time, the proportions of the fat and carbohydrate, and to a less degree of the protein, were varied and the effect on the flora noted.

16. *Centralbl. f. Bakteriöl.*, Abt. 1, O., 1912, 62, p. 432.

17. *Jour. Am. Chem. Soc.*, 1913, 35, p. 1201.

The studies of Herter and Kendall,⁵ on the changes in the intestinal flora of monkeys in response to various diets, were the first attempts in the determination the nature and degree of the substitution of bacterial types induced by abrupt variations in the chemical composition of the food. In these experiments, the animals were fed for one or two weeks on a diet consisting almost entirely of protein and then shifted abruptly to a diet consisting very largely of carbohydrate. On the protein diet the flora consisted largely of gram-positive proteolytic or peptolytic bacteria, many of them spore bearing, also many of the colon and coccal types. After a few days of feeding with the carbohydrate diet, this markedly putrefactive flora underwent a rapid change to one of a fermentative type, resembling strikingly that of a human nursing in the predominance of the *bacillus acidophilus* and the *bacillus bifidus* types. The change of flora apparently was mainly due to the influence of the carbohydrate. Recently Rettger and Horton¹⁸ have published a somewhat similar study with white rats as the subjects. The intestinal floras of rats on an ordinary mixed diet were compared with the flora of rats on a special diet, which was being used in nutrition experiments by Osborne and Mendel and which consisted of a pure protein (18 percent), protein-free milk (28 percent), starch (29 percent), and lard (25 percent). On the special diet, the flora became simplified and consisted largely of gram-positive bacteria of the *acidophilus* and *bifidus* groups with a reduction in the number of colon bacilli.

Bacteriological studies of this character have been extended to man only in a very few instances. Herter⁵ observed that, after the addition of 100-200 gm. of cane sugar daily to the ordinary mixed diet of an adult normal human subject, the feces became soft, acid in reaction and odor, and the numbers of bacteria of the *acidophilus* type increased. MacNeal and his associates conducted an elaborate study of the bacterial elements in the stools of twelve normal adults placed on a definite, weighed diet, but apparently no attempt was made to correlate changes in the flora with chemical variations in the food administered. Kendall has recently attempted to change the "obligate dysentery flora" of infants, consisting of the *bacillus dysenteriae*, the *bacillus coli*, and *streptococcus*, which is potentially putrefactive, to one of a purely fermentative type through the feeding of lactose. In his favorable cases, he noted a gradual disappearance of these gram-negative forms and of *streptococci* associated with the gradual appearance of the *bacillus bifidus* and the *bacillus acidophilus*.

DISCUSSION OF CASES

In Table 1 are detailed the changes in bacterial types which may occur as the result of liberal carbohydrate feeding in typhoidal patients with an initial favorable "facultative" intestinal flora. In these and certain other patients, the first bacterial response to a diet of 2,000-3,000 calories with the carbohydrate portion amounting to 250-300 gm. was a great increase in the numbers of colon bacilli or *streptococci*, or, in some instances, of both types. Within two weeks, however, the *bacillus acidophilus* had increased enormously until, in one case, it apparently outnumbered other viable bacteria about 500 to 1. This marked increase of the *bacillus acidophilus* is shown, not

only by a comparison of the counts from the anaerobic lactose agar plates with those from the aerobic gelatin or sugar-free agar plates, but also by the vigorous growths obtained in the acetic acid glucose broth tubes and by the direct counts from the gram-stained fecal smears. With both the patients (John T. and Enrico P.) these smears had become markedly gram-positive within two weeks and 97 percent of the bacteria seen were of the acidophilus type (John T. and Enrico P., Table 1). In the case of Enrico P., an examination of the sediment from the lactose broth fermentation tube showed that the bacillus bifidus was well represented in the stools, and it is possible that some of the bacteria classified as *B. acidophilus* in the count from the fecal smear were actually *B. bifidus* without the swollen or bifid end. The results with these patients prove that, even in adults and in conjunction with an inflammatory condition of the intestinal tract, the flora may be changed through a liberal carbohydrate diet to such a degree that, in many respects, it closely resembles the fermentative and comparatively non-gas-producing flora of the nursing.

In this series of cases, there was none exhibiting in the first examinations a putrefactive flora of the extreme type. There were, however, several examples of a fairly marked putrefactive type, observations on three of which are detailed in Table 4. Such of those cases as were examined soon after admission to the hospital did not reveal the presence of the bacillus acidophilus in the stools; on the other hand, bacteria liquefying gelatin and Loeffler's serum medium were apparently abundant, the sediments in the lactose bile fermentation tubes were putrefactive in type and a comparatively large number of spores developed on the anaerobic lactose agar plates. One of these patients (James C.) died soon after admission to the hospital and another (Thomas F.) could not be placed on more than a moderately high calory diet during the first two weeks in the hospital. As the amount of carbohydrate was gradually increased, the intestinal flora of this patient changed from a putrefactive to a moderately fermentative type. In the case of Emil C. (Table 4) altho the issue is somewhat confused by the alternation at one stage of a high fat with a high carbohydrate diet, practically the same degree of change is revealed. In Table 3 are detailed the results with two patients (Howard F. and Charles F.) of definite, but somewhat less, putrefactive tendencies than the ones just mentioned. Neither of them could tolerate liberal feeding for nearly a month after admission to

the hospital. On the comparatively moderate amounts of carbohydrate which were given them during the first three weeks, there was, at first, some increase in the numbers of colon bacilli and streptococci followed by a moderate multiplication of the bacillus acidophilus, as indicated by the increased growth in the acetic acid glucose broth tubes and the differential counts from the fecal smears. Toward the end of the period of the observation, the amount of carbohydrate fed was greatly increased, but even under these conditions there was nothing like the substitution of floras which was revealed so strikingly for the two cases reported in Table 1.

The increase in the acidophilus types, which are believed to multiply principally in the large intestine and which are dependent upon carbohydrate in their metabolism, would seem to indicate that the amount of carbohydrate given to most of these cases was sufficient to encourage the bacilli of the colon group to exercise their fermentative, rather than their putrefactive, propensities. Du Bois¹⁹ determined, in a study of the food absorption in typhoid fever, that when carbohydrate was given in amounts under 300 gm. a day, it was present in the stools only in traces, if at all, whereas, when amounts over 300 gm. were fed, the stools sometimes contained 2-3 gm. of reducing bodies. These results, it should be observed, were obtained with material which had passed through the whole length of the intestinal tract and had been subjected not only to absorption but to the action of fermentative bacteria. It seems altogether probable that with the large amounts of lactose fed in addition to other carbohydrates some of this sugar may have reached the level of the colon in sufficient quantity to exert a sparing action in that locality on the bacterial nitrogen metabolism. I have found that it is necessary to add only very small amounts of dextrose to Dunham's peptone medium in order to prevent the formation of indol by colon bacilli. Seeding tubes with the bacillus coli communis and also with the bacillus coli communior, indol had formed in medium containing 0.1 percent of dextrose, but not in that containing 0.2 percent. This same result was obtained after 3 days' incubation at 37 C. and also after seven days' incubation. It is evident, then, that the presence of only slight amounts of a usable carbohydrate are necessary in order to prevent the peptolytic action of the bacillus coli.

In six patients, the initial flora, that is, the one observed before carbohydrate had been given in liberal amount or at most for not more than a few days, was markedly fermentative in type. The bacterial findings in four of these cases are given in detail in Table 2. The dominant bacteria in the stools were from the start of the bacillus acidophilus type and there was a notable absence of putrefactive organisms. Accordingly, there was present in these cases at the beginning of the attack a type of flora which was attained in certain patients, with an initial facultative type, only after two weeks or more of feeding with large amounts of carbohydrate. As might be anticipated in these cases, the high carbohydrate diet maintained and accentuated this purely fermentative and relatively non-gas-producing flora. It seems not unlikely that if perforation should occur in cases with a flora of this character, the results might be less disastrous than for patients harboring an intestinal flora of putrefactive propensities.

As far as the incidence of the typhoidal infection is concerned, there was no evidence that it was favored by one type of intestinal flora more than by another; this series of cases exhibited intestinal bacterial conditions in the early stages of the attack which ranged from the putrefactive to a benign type of fermentative flora. Of course, the conditions at the time of primary infection may only be surmised, altho there is no reason to suppose that they differed markedly from what was encountered on their admission to the hospital.

With most of the cases especially in the earlier stages of the feeding, the daily amount of fat in the diet ranged from 75-125 gm. The effect on the cultural results of a sudden shift to a diet containing a larger ration of fat apparently depended upon whether the carbohydrate allowance was simultaneously decreased or was maintained at a high level. If, after a fermentative flora had been established, the fat in the diet was increased to about 200 gm. and the carbohydrate reduced to the same amount or less, there occurred a reduction in the number of viable bacteria in the stool which generally included the colon types, but was especially striking as regards the aciduric types. In the case of Christian M. (Table 2) the bacillus acidophilus was apparently almost eliminated, but on a return to the high carbohydrate feeding, it had again regained its dominance within three days. It should be observed that the amount of protein was kept at the same level in the diet of this case and only the fat and carbohydrate were varied in amount. If the amount of fat was increased to about

200 gm. daily, but, at the same time, the carbohydrate was maintained at 300-400 gm., any reduction in the number of viable bacteria in the stools which might occur concerned alone the bacilli of the colon group and the numbers of acidophilus bacilli remained at a high level (Table 2, Anthony G., Table 1, John T.). During the convalescence of Enrico P., about equal amounts by weight of protein, fat, and carbohydrate were given for a period of ten days. This resulted in an almost complete elimination of the bacillus acidophilus and an increase in the numbers of colon bacilli, the appearance of the direct smear from the feces changing from a predominately gram-positive type to an equally markedly gram-negative one (Table 1). I intend to enquire farther into the effect of varying the amounts of fat and carbohydrate on the intestinal flora. My results thus far indicate that fat is not potentially related to carbohydrate from a bacterial standpoint as has been suggested by Kendall.³

The stools of patients on a high carbohydrate diet were almost always moist and semi-formed, whereas those from patients on a high fat diet were formed and generally rather dry. The question arises, then, whether the cultural results with the stools of patients on a high fat diet reflect the bacterial conditions in the lower ileum and colon or whether the inspissation of such stools in the passage through the lower colon and rectum was the factor responsible for the variations in the numbers of viable bacteria. As regards the colon bacillus, altho the increase and decrease of their colonies could not always be correlated with the degree of moisture in the movement, yet frequently a low count was associated with a rather dry formed stool as MacNeal and his associates found in their series of examinations. On the other hand, the inspissation of the stool seemed to bear no relation to the numbers of the bacillus acidophilus which might appear in the cultures, but their increase and decrease, as revealed in the stool examinations, were governed entirely by the relative and actual amount of carbohydrate in the diet. It is in fact well established that these aciduric bacilli withstand drying successfully. Accordingly, there are good grounds for the assumption that the numbers of the bacillus acidophilus revealed in the cultures constitute a true index of their numbers at the level of the intestinal tract which they frequent.

It seemed advisable to determine whether or not typhoid patients, given a purely milk diet, would exhibit changes in their fecal flora similar to those which were observed with patients on a high calory

diet with a liberal proportion of carbohydrate. Through the courtesy of Dr. Lewis A. Conner, I had the opportunity of examining bacteriologically the stools of three such patients at the New York Hospital. Two of these patients (Table 5, Joseph P. and Torello D.) were given daily an average of about 52 oz. of milk. This constitutes a liberal feeding of milk, but, nevertheless, is a low calory diet. This amount was probably equivalent to about 975 calories, of which the carbohydrate amounted to approximately 70 gm., the fat to 52 gm., and the protein to 45.5 gm. This amount constituted no more than one-third to one-half the amount usually given in the Coleman high calory diet, even in the early stages of the disease. It would seem unlikely that this allowance of carbohydrate would prove sufficient to encourage or maintain a benign fermentative flora, and such proved to be the fact. In one of these milk-fed cases (Table 5, Joseph P.) a distinctively fermentative flora was encountered at the beginning of the trial, but after four days of milk feeding the organisms of the bacillus acidophilus type had decreased greatly in numbers and in eight days they were almost entirely replaced by colon and streptococcus types. The fecal flora had thus undergone a transformation to one potentially peptolytic and indol-forming. As there was a history of a milk diet for this patient for two weeks before admission to the hospital, about 3 weeks were apparently required to bring about the marked change in the character of the flora. In the second of these cases (Torello D.), during fifteen days on a milk diet, the initial moderately putrefactive type of flora was transformed to a peptolytic, but not to a purely fermentative, type. Toward the end of the period, there was a marked increase in the number of streptococci. A much greater variety of bacteria was observed in the gram-stained smears and also on the culture plates seeded from the stools of these two milk-fed patients than was the case with patients fed liberal amounts of carbohydrate. In fact, the simplification of the flora, following a course of high carbohydrate feeding, was a very striking feature. On the milk diet, colon types which acted slightly or not at all on lactose were common, a state of affairs which suggests an explanation of the fact that greater volumes of gas were formed in the dextrose than in the lactose fermentation tubes. The stool examination of the third patient on the milk diet revealed the same general features as the other two. At the time of taking the specimen, she had been four days on the milk diet and there was, in addition, a history of milk feeding for one week before admission.

As far as typhoidal cases are concerned, the bacteriological examination of these stools would seem to indicate that a daily diet containing such a small amount of carbohydrate as 70 gm. with about two-thirds as much each of fat and protein is not a combination which will encourage the development in the adult of a non-indol forming intestinal flora. On the other hand, the best results were attained, as regards the development and maintenance of a fermentative flora, when carbohydrate to the amount of 300-400 gm. was given daily. It is manifestly impossible to feed this amount of carbohydrate when milk alone constitutes the diet.

Altho the number of cases examined was too few to offer more than a suggestion, it is of interest to note that with five of six patients exhibiting a markedly fermentative flora, with the bacillus acidophilus dominant either before a diet containing much carbohydrate had been given at all or for not more than a few days, the infection ran a comparatively mild and short course. With the sixth patient exhibiting such a flora (Morris S., Table 2) altho the febrile period extended through two relapses, the patient's state of nourishment remained very good and convalescence was rapid. On the other hand, patients who suffered the severer attacks of the fever were those who harbored an initial flora of putrefactive tendency. Herter observed that individuals in middle life with an intestinal flora of the adolescent fermentative type were usually well nourished, free from nervous disturbances, and possessed of much reserve energy. When such individuals contract typhoid fever it seems not unlikely that their probable lifelong freedom from the continued absorption of the toxic products of a putrefactive fecal flora enables them to withstand the systemic invasion of the typhoid bacillus with greater success than can individuals who have been subjected to auto-intoxication for many years. I do not wish to lay stress on this suggestion at this time but it is well known that attacks of typhoid fever among children, in whom as a class auto-intoxication of intestinal origin is not a frequent factor, are often mild.

BACTERIAL OBSERVATIONS

Typhoid Bacillus.—A survey of the tables shows that the typhoid bacillus was isolated less frequently from this series of cases on the high calory diet than has been reported from certain other groups of cases on a less liberal diet. From a few patients, this bacillus was not isolated at all in examinations of the stools conducted at intervals dur-

ing almost the whole course of the fever and through convalescence. In almost every examination, from three to five Endo plates of the large size were made from graded dilutions of the stool and every suspicious colony was subjected to differential tests. In this procedure, a slant of Russell's double sugar medium was seeded from the colony in question and, if the growth and color reaction proved at all characteristic of the bacillus typhosus, the identification was established through agglutination tests. In certain instances where the typhoid bacillus was not recovered from stools with the Endo plate method, it was isolated by Dr. Oscar Teague, using a selective plating method. These comparative tests were carried out on only a comparatively few stools, and where success was attained the fact is indicated in the tables by an asterisk. Pratt, Peabody, and Long²⁰ observed no difference in the frequency of the isolation of the typhoid bacillus from the stools of patients on a milk diet and those on a more liberal diet, the amount and constitution of which is not definitely stated. A possible explanation of the infrequency of the isolation of typhoid bacilli from the stools of these patients on the high calory diet may be found in an unusual degree of acidity in the lower ileum and the colon, arising in that locality through the bacterial splitting of the large amounts of carbohydrate. The typhoid bacilli, eliminated from the gall-bladder, in their passage through the intestinal tract would probably find this environment unfavorable and the survivors might be so few as to be crowded out on the Endo plates by colonies of the ordinary intestinal bacteria. In this connection, it may be mentioned that Kendall noted a tendency of dysentery bacilli to disappear from the stools of infants suffering from summer diarrhea when lactose was fed in quantity sufficient to change the flora from a putrefactive to a fermentative type.

Streptococci.—Without regard to the general character of the flora and the nature of the diet, streptococci were present in considerable numbers in most of the stools. Streptococcus is apparently an organism which can adapt itself readily to changing and diverse conditions. On a liberal carbohydrate diet, streptococci generally increased rapidly during the first week or so but were largely replaced later by the bacillus acidophilus (Table 1). With a milk diet, the streptococci increased rapidly and continued to be present in large numbers in the stools. On the Endo plates, the streptococcus colonies ranged

from white to a deep scarlet. The white or pink colonies generally outnumbered the red and, by cultural tests, were found either to be raffinose fermenters and, hence, possibly to be grouped with the streptococcus salivarius, or to conform to the general type of the streptococcus lacticus. Certain of the deep red streptococcus colonies, which were subjected to differential tests, were found to be mannite splitters and in other respects to resemble the type, *Str. fecalis*.

Bacillus Acidophilus.—Rahe²¹ has investigated the biological characteristics of the bacillus acidophilus strains recovered from the stools of these typhoid cases and also cultures isolated from other sources. He has determined that the bacillus acidophilus group may be separated from the bacillus bulgaricus group, in a measure, through the type of colony formation on glucose oleate agar, but especially by the fact that the bacillus acidophilus ferments maltose whereas the bacillus bulgaricus is not able to split this sugar. The bacilli of the bacillus acidophilus type, according to his findings, may be further divided into two groups: those which clot milk within six days at 37 C., and those which do not clot milk within that period, and probably not at all. In a study of the strains of the bacillus acidophilus isolated from these typhoidal stools, it was found that in instances where the first examination from a patient disclosed large numbers of the bacillus acidophilus in the stool or where this bacillus became the dominant organism as the result of feeding large amounts of carbohydrate, the bacillus acidophilus present belonged in his Group 2, which clots milk readily, whereas cultures isolated from cases in which bacillus did not increase markedly as the result of such feeding belonged in his Group 3, which does not clot milk within six days' incubation.

Other Bacteria.—The bacillus pyocyaneus was found in the stools during the earlier stages of the disease in a few of the cases. These cases included both mild and severe types. This bacillus, in each instance, disappeared from the feces after the patient had been fed for a time with a high carbohydrate diet. No proteus-like colonies were observed on any of the plates. As has been mentioned, spores occurred in relatively small numbers in the most of the stools. In a number of the patients to whom carbohydrate was given in large amount, practically no spores developed on either the aerobic or anaerobic plates, but following a high-fat diet colonies developing from spores appeared in increased numbers on the aerobic plates. These were mostly thin,

dry, spreading, and frosted in appearance. Such spores as developed on the anaerobic plates were largely of the *bacillus aerogenes capsulatus* type. Spores of the *bacillus putrificus* type were rarely observed in the smears from the feces. Louis-Melikov²² has emphasized the supposed part played by anaerobic proteolytic bacilli, resembling the *bacillus perfringens*, in the production of the intestinal lesions. He has designated them "satellite bacilli." In our series of cases, neither the cultures nor the examinations of the smears has revealed unusual numbers of the spore-bearing bacilli of the type he describes. In the smears from the stools of some of the patients, many yeasts were observed. In one instance (Table 2, Morris S.), the frothy character of the stools was apparently due to them.

SUMMARY

In a series of typhoid cases, the intestinal flora as revealed by examinations of the stools soon after the admission of the patients to the hospital was not uniform or specific in type, but exhibited variations that might be observed in a series of supposedly normal individuals. Apparently, the character of the intestinal flora is not a factor concerned in the determination of susceptibility to typhoidal infection.

On a diet consisting of a daily average of 50-100 gm. of protein, 75-100 gm. of fat, and 250-300 gm. of carbohydrate, including lactose, the intestinal flora tended to become simplified in regard to the variety of bacterial types observed in direct gram-stained smears from the feces and in cultures and to become converted into a fermentative type in which the dominant organism was *Bacillus acidophilus*. The degree of this transformation was dependent largely upon the type of flora which was present at the onset of the disease. When the flora showed a definite putrefactive tendency, the change did not extend farther than the elimination of the obligate putrefactive organisms and a moderate development of the aciduric types; while, with a more favorable initial flora, the change was of so radical a type that the stools finally resembled those of the first few years of normal infancy in the dominance of the *bacillus acidophilus* and even in the presence of the *bacillus bifidus*. Such a flora is not an obligate fermentative one and under the conditions of feeding is not capable of forming indol. Observations on a few patients given a purely milk diet indi-

cated that this food alone does not bring about this transformation of the intestinal flora to such a degree as that which may follow a more liberal carbohydrate feeding.

Patients exhibiting an initial fermentative flora of the aciduric type adapted themselves more readily to the high calory diet of Coleman, as shown especially by a comparative freedom of distention, than did patients with floras of putrefactive tendencies; in such patients, the diseases showed a marked tendency to run a mild course.

The typhoid bacillus was isolated from the stools of these patients on a high calory diet less frequently than has been reported for other series of cases in which the feeding was less liberal.

The Journal of Infectious Diseases

PUBLISHED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. 16

March, 1915

No. 2

THE MECHANISM OF PHAGOCYTOSIS *

G. L. KITE AND W. B. WHERRY

(From the Marine Biological Laboratory, Woods Hole, Massachusetts)

INTRODUCTION

It can hardly be denied that for some years we have been under the impression that the phagocytic activity of certain metazoan body cells is analogous to that exhibited in the selective feeding reactions of many protozoa. The theory of Metschnikoff is based apparently on numerous observations which give support to such a conception. The writings of his pupils, Bordet, Burnett, and others, show clearly that they too have been hampered by such views. The very *in vitro* technic by which we are to study the activity of the phagocytes in taking up bacteria is based on this idea, else why incubate the mixture? It may be possible that some of the recorded observations of previous workers have been correctly interpreted, but we feel that in the light of the following experiments it will be necessary for us to alter our conception of the mechanism of phagocytosis.

Instead of the prevalent conception, we suggest that foreign particles, as carbon, carmin granules, etc., are "taken up" by leukocytes because the latter, as amebae of the limax group in the trophozoite stage, have sticky surfaces. The cell in its movements crawls over or

* Received for publication November 4, 1914.

against such particles, which in turn adhere to the cell surface and then are carried into the interior by cytoplasmic streaming currents. That amebae of the limax group, while in the trophozoite stage, are provided with sticky surfaces was noted by Sellards,¹ while working with the Barber pipette, and independently by Kite,² who further observed that this ameba (*Vahlkampfia* sp.) was not sticky while in the motile, flagellated stage. Furthermore, Kite³ has noted that polymorphonuclear leukocytes also show a similar glass-adhesiveness under in vitro conditions, and he believes that different cells show considerable differences in degree of adhesiveness.

The fact that leukocytes are only barely able to take up many kinds of bacteria under in vitro conditions without the aid of something present in the body fluids which acts upon the bacteria rather than upon the phagocytes is now well known through the work of Wright, Hektoen, and others, and we believe that our experiments show clearly that "opsonins" act by making the bacterial membrane or capsule more sticky, so that the bacteria have a greater tendency to adhere to the surface of leukocytes which comes into contact with them. Some of the classical experiments of in vitro phagocytosis are here reproduced by forcing leukocytes through a bacterial emulsion by centrifugal force, and by less violent agitation; in fact, we have been unable to make a mixture of "opsonized" bacteria and leukocytes without getting some "phagocytosis."

The amount of adhesion in a mixture of bacteria, leukocytes, and unheated serum must depend, it seems to us, on the relative stickiness of the bacteria and the leukocytes.

The stickiness of the leukocytes we used in our in vitro experiments varied widely. For example, in Experiment 17, where cocci and unheated serum were incubated separately from washed leukocytes suspended in unheated serum and then the two suspensions agitated together, there was a marked variation in the number of bacteria taken up by individual leukocytes. In the fifty leukocytes counted in the smear, three contained no cocci, ten, 16-20 cocci, and the rest varied between 3-14 cocci. Here, an eosinophil containing 19 cocci and a large mononuclear with 6 cocci were noted. These were leukocytes from normal peripheral blood. It seems probable that this variation will be found to be much more marked in exudates and may

1. Philippine Jour. Sc., Series B, 1911, 23, p. 81.

2. See Wherry: Arch. f. Protistenk., 1913, p. 77.

3. Jour. Infect. Dis., 1914, 15, p. 319.

reach such a degree as to allow the adhesion of some bacteria without opsonization. In this connection, we will cite the counts on 100 cells in the osmic preparation in Experiment 9, where washed aleuronat frog's leukocytes and the potato bacillus were agitated together. This count showed sixty-six leukocytes with no bacilli; twenty-six, with 1-10 bacilli; three, with 15-16 bacilli; five, with 20-43 bacilli, and, as shown under the details of the experiment, certain clumps of leukocytes often showed a considerable degree of adhesion. The argument which might be invoked against the results of Experiment 17, that there was not enough opsonin present to treat all the cocci or that they were treated unequally, can hardly be used against Experiment 9 unless it can be shown that opsonin comes from the leukocytes. Similar observations by Tunncliff on the phagocytosis of the bacillus fusiformis and by Davis on the phagocytosis of influenza-like bacilli by washed leukocytes is mentioned by Hektoen.⁴ Numerous workers have called attention to the variation in phagocytic power shown by leukocytes collected from normal and diseased animals. This work has been exhaustively reviewed by Hektoen.⁵ We will refer here only to the observations of Rosenow,⁶ showing that pneumonic leukocytes are more active in their phagocytic power than leukocytes from normal blood. He also noted that such leukocytes "are more actively phagocytic for certain rare strains of pneumococci in the absence of serum."⁷ With regard to the greater stickiness of leukocytes in exudates, Tunncliff⁸ showed that the leukocytes in aleuronat exudates were more active phagocytically than the leukocytes obtained from the blood of the same animals.

We believe that Experiments 8, 10, and 11, made with a minimum of agitation, are very significant, for here practically just as dense suspensions of bacteria were used as in the other experiments, and yet they yielded comparatively low counts. The two experiments on direct observation of the mechanism of phagocytosis, under *in vitro* conditions, confirm the results of our agitation experiments so far as the actions of heated and unheated sera are concerned.

Finally, it is interesting to note that McKendrick⁹ in a theoretical and mathematical consideration of this subject concluded, in part: "the phenomenon of phagocytosis can be satisfactorily treated from

4. Jour. Am. Med. Assn., 1906, 46, p. 1407.

5. Ibid., 1911, 57, p. 1579.

6. Jour. Infect. Dis., 1906, 3, p. 683.

7. Ibid., p. 689.

8. Tr. Chicago Path. Soc., 1911, 8, p. 208.

9. Science Progress in the Twentieth Century, London, 1914, 8, p. 497.

the physical point of view as a random interfusion between two perfectly intermixed systems of particles, each of which is evenly distributed, in which ingestion takes place when individuals of opposite type have collided."

TECHNIC

For the most part, we have followed the regular procedure as described by Wright, with the exception that in most of the experiments the bacteria and leukocytes have been kept separated at various temperatures and then agitated together by running the mixture back and forth a given number of times in a standardized capillary pipette, as recommended by Wright, followed by the immediate preparation of smears. The smears were fixed with methyl alcohol and stained with aqueous toluidin blue (10 mg. to 100 c.c. distilled water). We were inclined to believe that the further agitation of the mixture during the process of making the blood films increases the degree of phagocytosis. We have not demonstrated this, but, on the other hand, we have some evidence that some of the best cells are lost during the process of smearing. In order to control the smeared preparations, we made wet-fixation films as follows:

Perfectly clean slides are placed in moist chambers (Petri dishes) and 5 or 6 drops of Ringer's solution placed on each slide which, if clean, will allow the fluid to spread immediately over the surface. These slides should be prepared shortly before they are to be used. Then, when the mixture has been made, a few drops of it are placed in the Ringer's and distributed over the surface of the slide and throughout the Ringer's by tipping the Petri dish two or three times. The leukocytes are then allowed to settle for five to ten minutes. The Petri dish is then placed on the flat surface of a block of ice and two or three minutes later three or four drops of 2 percent osmic acid are placed within the Petri dish beside the preparation. Fixation for ten to fifteen minutes is followed by thorough washing with absolute followed by 95 percent alcohol and water. The affinity of such (osmic) preparations for toluidin blue is markedly increased, and staining must be practically instantaneous in order to get preparations in which the bacteria can be counted with any degree of accuracy. If too deeply stained, they must be decolorized and restained. Such preparations have many advantages over the ordinary smears. The leukocytes often retain to a greater or less degree their normal spherical contour. One often encounters leukocytes which plainly show that the bacteria are adherent to the surface of the cell and have not yet been carried into the deeper layers of the cytoplasm. In properly stained and differentiated specimens, there is no difficulty in counting the bacteria even tho they occur at different depths in the cell. Here, too, one often encounters large and small mononuclears and eosinophils which have cocci adherent to them. In fact, with proper differentiation one can see a considerable number of bacteria sticking to the lymphocytes.

Most of our experiments were done with the *staphylococcus pyogenes aureus*, isolated about a month previously from a severe infection of the finger. The

cultures were grown at the temperatures given for given lengths of time on +1 agar slants aerobically. Suspensions were made in sodium chlorid solution or in Ringer's of the concentrations mentioned later and centrifuged to remove the coarser clumps. In Experiments 9, 10, and 20, an actively motile, non-sporogenic bacillus (*B. fluorescens liquefaciens*?) isolated from potato was used.

The human leukocyte suspensions (including many erythrocytes) were prepared by collecting blood in 1.5 percent sodium citrate followed by washing three times with 0.85 percent sodium chlorid solution (Experiments 1 and 2) or Ringer's 0.69 percent (Experiments 3-10) or Ringer's 0.86 percent (Experiments 11-22). The 0.69 Ringer's had the following composition: NaCl 0.65, KCl 0.02, CaCl₂ 0.025 percent; the 0.86 percent Ringer's, NaCl 0.8, KCl 0.02, CaCl₂ 0.02, NaHCO₃ 0.02 percent.

In order to make the various experiments as comparable as possible, the leukocytes and serum from one of us was used throughout the series, and this one made all the mixtures and agitations.

In determining the degree of phagocytosis, the average number of bacteria in fifty polymorphonuclear or transitional leukocytes was counted with a Zeiss 1.8 mm. apochromatic lens, No. 6 ocular, and a bright artificial light.

EXPERIMENTS

The following experiments show that on separate incubation followed by agitation marked "phagocytosis" is produced.

Experiment 5.—(1) Cocci in 0.69 Ringer's, (2) Same suspension of cocci with an equal volume of twenty-four-hour-old human serum, and (3) washed, human blood suspended in serum were incubated separately for fifteen minutes at 34 C. Nos. 1 + 3 and 2 + 3 were mixed in small tubes with capillary pipettes five times and slowly centrifuged once; they were mixed again five times, and smears and osmic preparations were made. Smears 1 + 3 showed 23 cocci per leukocyte; Smears 2 + 3 showed 41 cocci per leukocyte; the Osmic 1 + 3 showed 32 cocci per leukocyte; in the Osmic 2 + 3 the cocci were too crowded to count accurately. It will be noted that in Nos. 1 + 3 twice as many cocci were present as were in Nos. 2 + 3, and that here opsonization took place during the mixing and centrifuging. While counting the fifty cells in Smears 1 + 3 two eosinophils were encountered containing 8 and 12 cocci, respectively; likewise, in the osmic preparation eosinophils contained 6, 14, and 9 cocci; in Smears 2 + 3 eosinophils contained 32, 33, and 24 cocci.

Experiments 8, 10, and 11 show the effects of a minimum degree of agitation.

Experiment 8.—Washed (0.69 Ringer's) human corpuscles were suspended in an equal volume of twenty-four-hour-old serum. (2) A fairly dense emulsion of cocci in 0.69 Ringer's was diluted with an equal volume of the serum. Equal volumes of Mixtures 1 and 2 were then placed in a short 15 mm. test tube—the cocci being gently dropped into the blood and given a rotary shake two or three times and then incubated at 39 C. for 30 minutes. The precipitated sediment was then removed with a 3 mm. glass tubing in order to avoid the churning of the capillary pipette, and smears and osmic preparations were made. The smears showed 1.2 cocci and the osmic 1.9 cocci per leukocyte.

Experiment 10.—The technic used in Experiment 8 was used with the substitution of the actively motile bacillus from potato. The mixture was incubated for fifteen minutes at 36 C. The smears showed 7 bacilli per leukocyte and the osmic 4.

Experiment 11.—The technic was that used in Experiment 8, excepting that 0.85 percent NaCl was used. The mixture was incubated for twenty minutes at 38 C. The smears showed 7 cocci per leukocyte and the osmic 4.

The following agitation experiments show the effect of dilution of the staphylococcus emulsion on the degree of adhesion when the leukocytes and bacteria are incubated separately and then mixed and smeared. The serum in these three experiments could act on the bacteria only during the few seconds taken in mixing—hence the comparatively low counts.

Experiment 13.—The coccus emulsion was diluted to one-half, one-tenth, and one-twentieth the density of a twenty-four-hour typhoid broth culture with 0.86 percent Ringer's. The washed corpuscles were suspended in an equal volume of eighteen-hour-old serum, incubated for fifteen minutes at 38 C., and again mixed before mixing with the bacteria. Equal volumes of the bacterial emulsions and leukocyte-serum mixture were mixed five times in capillary pipettes of approximately the same size and then smeared. The one-half dilution gave 3 cocci, the one-tenth 0.02, the one-twentieth 0.01 cocci per leukocyte. The osmic preparations gave 2, and 0.02. The last dilution was not counted.

Experiment 14.—The cocci had been kept seven days on + 1 agar at 21 C. The washed leukocytes in eighteen-hour-old serum were incubated at 37 C. for fifteen minutes. The coccus emulsion was diluted one-half, one-fourth, and one-eighth its original density and the mixing was performed with short, three-inch, capillary pipettes. Otherwise, the technic was similar to that used in Experiment 13. The one-half dilution gave 2.9 cocci, the one-fourth 1.1 cocci, and the one-eighth 1 coccus per leukocyte. The osmic preparations were not counted.

Experiment 15.—This duplicates Experiment 14, excepting that the forty-eight-hour-old serum was incubated with the leukocytes for fifteen minutes at 38 C. and that the coccus emulsion was finally diluted to one-half and one-fourth its original density. The mixture was churned ten times in six-inch capillary pipettes before smears were made. The one-half dilution gave 2.4 and the one-fourth 1.2 cocci per leukocyte.

Experiment 16 clearly shows that if more time is given for the action of the serum upon the bacteria more of the latter adhere to the leukocytes.

Experiment 16.—The serum was fresh—three hours on ice. The coccus suspension from Experiment 15 was used. Two volumes of this suspension were mixed with one volume of serum and kept at 21 C. for thirty minutes. A similar mixture of cocci and serum was kept at 21 C. for fifteen minutes and then at 38 C. for fifteen more minutes. Equal volumes of these two bacterial suspensions were then churned ten times in six-inch capillary pipettes with similar volumes of washed leukocytes which had been incubated in serum for fifteen minutes at 38 C. The 21 C. test gave in the smear 6.7 cocci and in the

osmic 6.6 cocci per leukocyte. The 21 C. plus 38 C. test gave in the smears 6.4 cocci and in the osmic 6.4 cocci per leukocyte. The final dilutions of the coccus emulsions were here nearly one-half the original density and the figures show that with the greater time for opsonization more than twice as many cocci adhered.

During the counting of the 200 polymorphonuclears in this experiment, thirteen large mononuclears were seen with from 3-6 adherent cocci; twelve lymphocytes with from 1-9 adherent cocci and eight eosinophils with from 1-5 adherent cocci.

That the usual result produced by heating serum can be demonstrated by churning separately incubated mixtures of bacteria and serum and serum and leukocytes is shown by Experiment 18.

Experiment 18.—The cocci from a forty-eight-hour-old culture were suspended in 0.86 Ringer's. The serum was separated for forty-eight hours on ice, and half of it heated for fifteen minutes at 55 C. Equal volumes of the heated and unheated sera and of the coccus suspension were thoroughly mixed and kept at 21 C. for twenty-eight minutes. Likewise, equal volumes of thoroughly agitated washed leukocytes in heated and unheated sera were incubated at 38 C. for fifteen minutes. Four mixtures were then made by churning ten times in six-inch capillary pipettes and smears and osmic preparations made at once:

The cocci in unheated serum plus leukocytes in unheated serum gave in the smears, 9 cocci, and in the osmic, 8 cocci per leukocyte; the cocci in heated serum plus leukocytes in heated serum gave both in the smears 0.1 coccus per leukocyte and in the osmic 0.1; the cocci in the heated serum plus leukocytes in unheated serum gave in the smears 2 cocci and in the osmic 0.6 coccus per leukocyte; the cocci in unheated serum plus the leukocytes in heated serum gave in the smears 9.9 cocci and in the osmic 9.6 cocci per leukocyte.

The following experiments were performed to test the influence of temperature on the "opsonization" of bacteria by normal serum.

Experiment 19.—The cocci from a forty-eight-hour-old culture were suspended in 0.86 Ringer's and were chilled in the ice-box at 11 C. The serum which was three hours old was also chilled to 11 C. Three mixtures of equal volumes of bacteria and serum were then made; one was kept for forty-five minutes at 11 C.; another at 21 C. for thirty minutes and then for fifteen minutes at 11 C.; the last was kept at 36 C. for fifteen minutes and then at 11 C. for thirty minutes. Then, equal volumes of these suspensions were separately churned ten times in six-inch capillary pipettes with washed leukocytes which had been suspended in serum at 36 C. for fifteen minutes. Those kept at 11 C. gave in the smears 9.7 and in the osmic 8 cocci per leukocyte; those at 21 C. gave in the smears 9 and in the osmic 11 cocci per leukocyte; those at 36 C. gave in the smears 11 cocci and in the osmic 8.7 per leukocyte.

As it seemed rather surprising that "opsonization" should occur about as well at 11 C. as at 36 C., lower temperatures were tried as follows:

Experiment 20.—The potato bacillus in 0.86 Ringer's, and two-hour-old serum were kept in separate tubes in crushed ice at 1-2 C. In a few minutes, equal volumes of the two were mixed and one portion kept at 1-2 C. and the other

at 21 C. for thirty minutes. Washed leukocytes suspended in serum were kept at 37 C. for fifteen minutes. Equal volumes of the two suspensions and of the leukocytes were then churned twelve times in six-inch capillary pipettes and smeared. That kept at 1-2 C. gave 2 bacilli, and that at 21 gave 9 bacilli per leukocyte.

Experiment 21.—The procedure of Experiment 20 was duplicated, excepting that a suspension of a twenty-four-hour-old culture of cocci was used and in this experiment the lower temperature was kept at 1 C. That kept at 1 C. gave 3 cocci while that kept at 21 C. gave 10.7 cocci per leukocyte. The osmic preparations were allowed to settle for too long a time (about twenty minutes) at 21 C. and when examined showed too much intracellular digestion for accurate counting. These preparations were similar to those encountered before in Experiment 6 and are particularly interesting in the fact that they demonstrate that the cocci are actually rolled into the cytoplasm of the cell and digested there. In many instances, cells were found with the intracellular cocci appearing as mere shadows whereas the cocci, still adherent to the surface of the leukocyte, appeared normal and stained intensely.

Only a single experiment was done on the possible influence of temperature on the leukocytes, and this experiment is not satisfactory so far as the lowest and highest temperatures are concerned, as precautions were not taken to collect and wash the leukocytes at a temperature other than that of the room, which was that of a warm day, 26 C.

Experiment 22.—The technic was that used in Experiment 20, excepting that the cocci plus serum were kept at 26 C. for thirty minutes and the three lots of the thoroughly mixed leukocytes were kept at 1, 26, and 38 C. for fifteen minutes. In the 1 C. experiment, the smears gave 9 and the osmic 13 cocci per leukocyte; in the 26 C. test the smears gave 10.8 and the osmic 13.9 cocci per leukocyte; in the 38 C. test the smears gave 11 and the osmic 14.7 cocci per leukocyte.

However, this experiment shows that it is hardly necessary to incubate the leukocyte serum mixture before agitation — as done by us in this series of experiments.

In the following experiment, frog's leukocytes were used.

Experiment 9.—Frog's leukocytes were collected by injecting a suspension of aleuronat into the peritoneal cavity, collecting some of the exudate in sodium citrate, and washing three times with 0.69 Ringer's. This suspension, containing many clumped leukocytes, was then mixed with a fairly dense suspension of the actively motile potato bacillus (in 0.69 Ringer's) and allowed to stand at 21 C. for thirty minutes. Then, some of this mixture was agitated thoroughly in a capillary pipette and smears and osmic preparations were made. The bacteria in 100 leukocytes in each preparation were then counted. The smears gave 2.8 bacilli and the osmic 3 bacilli per leukocyte. In the osmic preparations it was noted that the leukocytes which were massed together in clumps often showed a particularly high degree of adhesions, for example, the counts on the individual cells of five such clumps are as follows: (a) 28, 43, 25, 20; (b)

5, 23, three leukocytes too dark to count; (c) 0, 2, 28, two leukocytes too dark to count; (d) 0, 0, 0, 0; (e) 0, 13, 0, 0, 26. In a control microscopic preparation of this mixture, kept at 21 C., we were unable to detect any signs of phagocytosis altho some cells were watched for about an hour. Frequently, the actively motile bacilli bumped into the leukocytes but only occasionally stuck for a short time and then broke loose and escaped. This experiment shows clearly that some leukocytes, at least, are capable of having some bacteria adhere to them without the interaction of serum, provided that the bacteria are brought into contact with their surfaces with sufficient force.

DIRECT OBSERVATIONS ON THE MECHANISM OF "PHAGOCYTOSIS"

We have placed mixtures of leukocytes, serum and cocci on a modified Barber moist chamber¹⁰ at 23 C., and, by means of fine glass needle (1-2 μ) manipulated in the Barber pipette holder, have pushed cocci against leukocytes, and then, by traction on the cell, have seen that they adhere when unheated serum is used, and that they do not adhere when serum heated to 55 C. for fifteen minutes is used. Again, leukocytes have been dragged through such emulsions and in the one case they pick up many cocci while in the other they do not. Cocci picked up in this way seem to be rolled into the interior, and in the case of one cell we watched the digestive vacuoles form about the enclosed cocci which in less than an hour had dissolved and disappeared.

In the preparation containing washed leukocytes, heated serum, and cocci, we found one large mononuclear with eight or ten adherent cocci which could not be dislodged by dragging the leukocyte about. Other leukocytes which were dragged about in the same preparation did not have cocci adhere to them. This further supports the idea that some leukocytes are sticky enough for bacteria to adhere to them without the interaction of serum.

SUMMARY AND CONCLUSIONS

Following observations on the adhesive character of the surface of certain amebae and certain vertebrate leukocytes by Kite, experiments were undertaken which show that when separately incubated mixtures of leukocytes and serum and serum and bacteria are agitated together many of the bacteria stick to the leukocytes and are rolled into their substance. That the bacteria are actually within the cells is judged by observations on their digestion as shown both in fresh and in stained preparations. By this method, several of the classical experiments on

10. See Kite: Amer. Jour. Physiol., 1913, 32, p. 146.

phagocytosis have been repeated. Experiments with a minimum amount of agitation show that here "phagocytosis" is reduced.

We offer the suggestion that foreign particles, as carbon, are taken up by leukocytes because the latter have sticky surfaces; that bodies similar to many bacteria stick to leukocytes best in the presence of unheated serum because they adsorb something from the serum which makes them more sticky or are in some way rendered more sticky, and hence the chances of their adhering to the surfaces of leukocytes are increased. There is evidence that, even in the absence of serum, certain leukocytes are sticky enough to allow some bacteria to adhere. Preliminary experiments on the influence of temperature on the power of serum to make staphylococci sticky seem to indicate that this may occur as well at 11 C. as at 37 C., but that the action is diminished considerably at 1 C.

THE TECHNIC OF THE WASSERMANN REACTION

WITH REFERENCE TO THOMAS AND IVY'S METHOD OF COMPLEMENT DOSAGE AND TO THE MANAGEMENT OF NATURAL ANTISHEEP AMBOCEPTOR*

REUBEN OTTENBERG AND BLANCHE FRAZIER

(From the Department of Bacteriology, College of Physicians and Surgeons, and the Pathological Laboratory of Mount Sinai Hospital, New York City)

Thomas and Ivy¹ have recently proposed a method for increasing the delicacy of the Wassermann reaction. Their method consists of the use of the minimum amount of complement and amboceptor which will give complete hemolysis in the presence of the proper quantity of antigen and of (pooled) negative serum. They make no reference to the occurrence of natural antisheep amboceptor in the human serum which was added in titrating the complement. In attempting to test their method we have found that this may occasionally lead to error.

Many authors have referred to the frequent occurrence of natural antisheep amboceptor in appreciable amounts in human serum. The well-known modifications of Bauer,² as well as those of Hecht and Weinberg and others, take advantage of this and the substitutes for the Wassermann method, such as that of Noguchi, are devised chiefly to eliminate the error caused by it. The occasional occurrence of a great excess of such natural amboceptor is also well recognized.³ We have found in 2,158 tests that 955 sera (44 percent) had an appreciable amount of natural amboceptor and that 476 sera (21 percent) had sufficient to produce complete laking without further addition of amboceptor.

Thus, a number of negative sera pooled at random almost always have some natural amboceptor, and we have occasionally found that such pooled sera had so much natural amboceptor that complete hemolysis occurred without the addition of artificial amboceptor. It is obvious (from the law of inverse ratios of Morgenroth and Sachs⁴) that under these circumstances the complement titration would give too

* Received for publication November 13, 1914.

1. Am. Jour. Med. Sc., 1914, 148, p. 55.

2. Berl. klin. Wehnschr., 1908, 15, p. 843.

3. Kaliski: Arch. Int. Med., 1910, 6, p. 205.

4. Berl. klin. Wehnschr., 1902, 39, p. 817.

small a dose. If Thomas and Ivy's proposal is adopted, it is therefore necessary in every case to choose negative sera which are known to have little or no natural amboceptor.

Altho some workers pay no attention to natural amboceptor, a perusal of the literature and our own experience leave us no doubt that if the presence of natural amboceptor is disregarded a certain small percentage of false negative reactions is obtained. Some authors have undoubtedly exaggerated the proportion of such cases. Nevertheless, it is obvious that the occasional coincidence of a large excess of natural amboceptor with a small amount of so-called "syphilitic antibody" will inevitably lead to error. In spite of the many methods which have been proposed to obviate this difficulty there is none entirely satisfactory. The absorption methods are accurate but cumbersome and sometimes render sera anticomplementary (Sachs phenomenon). Some observers⁵ make a preliminary test, often called the "Bauer control tube," to determine the natural amboceptor and then add immune amboceptor only to those sera that need it.

The method we have adopted, which originated at Mount Sinai Hospital with Dr. D. J. Kaliski,⁶ is similar to the Bauer method, but simpler. It consists of the addition of non-sensitized sheep cells, after the preliminary incubation, to all the given tests and incubation for ten minutes in the water bath at 37.5 C. At the end of this time those tests which contain a considerable excess of natural amboceptor show complete laking in the control tubes,[†] and to these tests no amboceptor is added. To all the other tests, the usual amount of two units of amboceptor is then added, and all the tests are incubated for the full period. Thus, the addition of still more amboceptor to those tests that already contain an excess is avoided.

It must be borne in mind that even with this method those rather exceptional sera that have a very great excess of natural amboceptor may still give false negative results. These sera (recognized by rapid laking in all the tubes) are re-tested after absorption of the natural amboceptor.⁷

Some workers from the New York Department of Health attempt to surmount the difficulty by reading the results as soon as the antigen

5. Dexter and Cummer: *Arch. Int. Med.*, 1912, 9, p. 605.

6. *Arch. Int. Med.*, 1912, 9, p. 214.

[†] Two control tubes are used for each serum, the one containing a full dose of serum (0.1) the other a half dose (0.05). The presence of two or more units of natural amboceptor is shown by complete laking in both of these tubes.

7. Rossi: *Ztschr. f. Immunitäts.*, 1911, 10, p. 321; Jacobaeus, *Ibid.*, 1911, 8, 615.

and serum controls are laked. Thus, Olmstead⁸ says: "An excess of amboceptor may result in false negative reactions. If, however, the readings are made as soon as antigen and serum controls are completely hemolyzed, an excess of amboceptor makes little difference in results."

Our experience, however, has shown us that those sera which contain an excess of natural amboceptor always lake much more rapidly than the antigen control, which of course contains only two units of amboceptor.

CONCLUSIONS

If attention is not paid to natural amboceptor a certain, tho small, percentage of positive results will be overlooked.

If Thomas and Ivy's method of titrating complement is used, it is essential to select negative sera which have no natural amboceptor.

Aside from this criticism, we shall not attempt here to pass judgment on Thomas and Ivy's method, but our experience leads us to fear that, if their proposal to use a single laking dose of complement and amboceptor is adopted, the margin of safety may not be large enough to avoid occasional, non-specific complement fixation.

8. Med. Rec., New York, 1914, 85, p. 341.

A COMPARISON OF THE IMMUNIZING EFFECTS OF THE SUBCUTANEOUS AND INTRAPERITONEAL ADMINISTRATIONS OF TUMOR CELLS AGAINST THE GROWTH OF CAR- CINOMA IN MICE *

M. G. SEELIG AND MOYER S. FLEISHER

(From the Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis, Missouri)

In a previous communication¹ it was shown that the influence of a tumor on a second tumor, inoculated six days later, varied with the degree of so-called virulence of the tumor. The percentage of growing tumors was taken as the measure of virulence. The virulence was experimentally varied, in accordance with the method of Loeb, by exposing the tumor material in vitro to a temperature of 44 C. for varying periods of time. By this method, it was possible to use the same strain of tumor in all experiments and yet to have tumor material the virulence of which we could absolutely control. We found in these earlier experiments three types of growth after double inoculation: Simultaneous, or concomitant, growth, when both tumors grew in an approximately normal percentage of the animals (this was found when both tumors were virulent or both were of a medium degree of virulence); alternating (mutually exclusive) growth which occurred when two tumors of low virulence were inoculated, and in this case usually only one of the tumors grew, either the first or the second; inhibiting type noticed when a virulent, or medium virulent, tumor was inoculated first and a tumor of low virulence second, in which case the second tumor either did not grow or grew only in a relatively small percentage of the mice. The degree of the inhibition varied in direct proportion to the length of time the second tumor had been heated. There was also noted in these experiments a fourth type of growth, a stimulating type noticed when the growth of a tumor of medium or low virulence was possibly improved by the growth or inoculation of a tumor of medium virulence, and the beneficial effect appeared sometimes to affect the first,

* Received for publication November 19, 1914.

1. Loeb: *Centralbl. f. Bakteriol.*, 1912, 63, p. 450.

sometimes the second tumor. The existence of this type was at that time considered only probable as it had not yet been definitely proven.

This paper deals with the comparison of effects of subcutaneous and intraperitoneal tumor inoculations. In these experiments, the same adenocarcinoma of the mouse was used as in the former experiments¹ (Tumor 9 first transplanted by Loeb eight or nine years ago), when both the first and second tumors were inoculated subcutaneously.

THE GROWTH OF INTRAPERITONEAL TUMORS

The methods and technic used in these experiments were the same as used in the earlier experiments.¹ Before considering the results of our experiments on immunity it is necessary to compare the growth of a tumor inoculated intraperitoneally with that of one inoculated subcutaneously.

We find that unheated tumor material, or tumor material heated for either twenty-five or thirty minutes, grows in a smaller percentage of mice when inoculated intraperitoneally than when inoculated subcutaneously with material of similar virulence (Table 1). A heated or an unheated tumor, when inoculated intraperitoneally, grows in 60-70 percent of the animals in which similar material would grow if inoculated subcutaneously. When, however, five times the usual quantity of tumor material (375 mg.) is inoculated intraperitoneally, tumors develop in a slightly larger percentage of animals than after inoculation of the usual quantity. Intraperitoneal inoculation therefore is in our experiments less effective than subcutaneous inoculation. Therefore, our results regarding the percentage of growth of the intraperitoneal tumors differ from those of Krauss, Ranzi, and Ehrlich² and Paltauf.³

THE INFLUENCE OF A FIRST EITHER SUBCUTANEOUS OR INTRAPERITONEAL TUMOR ON A SECOND SUBCUTANEOUS TUMOR

When we compare the influence of a first, either intraperitoneal or subcutaneous, tumor on a second, subcutaneous tumor, inoculated six days later, we find that when an unheated tumor is inoculated first and either unheated tumor material or tumor material heated twenty-five minutes second, the immunizing effect of the less actively growing intraperitoneal (as compared to a subcutaneous) tumor is

2. Ztschr. f. Immunitäts., 1910, 6, p. 665.

3. Wien. klin. Wchnschr., 1909, 47, p. 1654.

greater than that of the first, subcutaneous tumor. The second, subcutaneous tumor grows in a smaller percentage of the animals when the first tumor is inoculated intraperitoneally than when inoculated subcutaneously. The effect of the first, intraperitoneal tumor on a second, unheated, subcutaneous tumor is the same as on a second, subcutaneous tumor heated either twenty-five or thirty minutes, but relatively the immunizing effect on the unheated tumor is greater.

TABLE 1
PERCENTAGE OF TAKES OF THE VARIOUS TUMORS

Subcutaneous Tumor	Intraperitoneal Tumor	Both First (1) and Second (2) Tumors Inoculated Subcutaneously	First Tumor (1) Inoculated Subcutaneously, Second (2) Intraperitoneally	First Tumor (1) Inoculated Intraperitoneally, Second (2) Subcutaneously
75 mg. unheated 82 %	375 mg. unheated 62 % 78 mice	(1) 75 mg. unheated 51 % (2) 375 mg. unheated 27 % 47 mice	(1) 375 mg. unheated 55 % (2) 75 mg. unheated 25 % 57 mice
75 mg. unheated 82 %	75 mg. unheated 53 % 118 mice	(1) 75 mg. unheated 86 % (2) 75 mg. unheated 76 % 27 mice	(1) 75 mg. unheated 77 % (2) 75 mg. unheated 19 % 43 mice	(1) 75 mg. unheated 45 % (2) 75 mg. unheated 24 % 64 mice
75 mg. unheated 82 % 75 mg. heated 25' 72 %	75 mg. unheated 53 % 75 mg. heated 25' 43 % 37 mice	(1) 75 mg. unheated 67 % (2) 75 mg. heated 25' 40 % 68 mice	(1) 75 mg. unheated 80 % (2) 75 mg. heated 25' 12 % 25 mice	(1) 75 mg. unheated 47 % (2) 75 mg. heated 25' 22 % 45 mice
75 mg. unheated 82 % 75 mg. heated 30' 55 %	75 mg. unheated 53 % 75 mg. heated 30' 34 % 53 mice	(1) 75 mg. unheated 73 % (2) 75 mg. heated 30' 21 % 37 mice	(1) 75 mg. unheated 84 % (2) 75 mg. heated 30' 21 % 38 mice	(1) 75 mg. unheated 43 % (2) 75 mg. heated 30' 20 % 41 mice
75 mg. heated 25' 72 %	75 mg. heated 25' 43 %	(1) 75 mg. heated 25' 80 % (2) 75 mg. heated 25' 60 % 28 mice	(1) 75 mg. heated 25' 45 % (2) 75 mg. heated 25' 30 % 54 mice
75 mg. heated 30' 55 %	75 mg. heated 30' 34 %	(1) 75 mg. heated 30' 80 % (2) 75 mg. heated 30' 26 % 21 mice	(1) 75 mg. heated 30' 26 % (2) 75 mg. heated 30' 61 % 74 mice

Thus, an unheated, subcutaneous tumor grows in 82 percent of the inoculated animals, but when it is inoculated six days after the inoculation of an intraperitoneal tumor, it grows in only 24 percent; a tumor heated twenty-five or thirty minutes grows subcutaneously in 72 percent or 55 percent of the animals, respectively, but when an intraperitoneal tumor has been inoculated first they grow in 22 percent and 20 percent of the animals, respectively. Thus, after the inocula-

tion of an intraperitoneal tumor the heated, and unheated, subcutaneous tumors grow in nearly similar percentages, whereas normally the unheated tumor grows in a considerably larger number of inoculated animals than does the heated tumor. We find in these cases that the growth is concomitant in type; the second tumor grows only when the first tumor grows (Table 2).

When an unheated tumor is inoculated intraperitoneally first and a tumor which has been heated for thirty minutes inoculated subcutaneously second we find no smaller inhibiting effect of the first intraperitoneal tumor than we would if the first tumor had been inoculated subcutaneously (Table 1). Thus, in the first case the tumor heated for thirty minutes grows in 21 percent, in the second case in 20 percent. Whether the results in these experiments are due to some factors which at present are not evident or whether attributable to coincidence, we cannot state, but even in this case we find the weaker, intraperitoneal tumor at least as active an immunizing agent against a second, subcutaneous tumor as is the much more strongly growing first, subcutaneous tumor.

When five times the usual quantity of tumor material is inoculated intraperitoneally first, a second unheated, subcutaneous tumor is very markedly inhibited, that is, when we compare the effect of the 375 mg. intraperitoneal first with the effect of 75 mg. subcutaneous first. However, the inhibition of the second unheated tumor by 375 mg. of tumor inoculated intraperitoneally is no greater than the effect of the usual 75 mg. inoculated intraperitoneally, even tho the former represents a more actively growing tumor (Table 1).

When the first, intraperitoneal tumor is heated the immunizing effect seems gradually to be lost; the growth of a second tumor heated for twenty-five minutes inoculated subcutaneously following an intraperitoneal tumor which had been heated twenty-five minutes, is better than when an unheated tumor is inoculated first intraperitoneally. When the first, intraperitoneal tumor is heated still longer, viz., thirty minutes, and a tumor heated for thirty minutes is inoculated second subcutaneously, we find that the second tumor has grown better than usual. Therefore, there seems to be a stimulating action produced by the first tumor on the second. On the other hand, the first, intraperitoneal tumor grows in a smaller percentage of the animals than normally, and it seems that the second, subcutaneous tumor has exerted an inhibiting influence on the first, intraperitoneal tumor. We find, therefore, in this case definite evidence of the beneficial

action of a tumor of low virulence on a second tumor of low virulence and also an approach toward the mutually exclusive alternating type of growth, inasmuch as the first intraperitoneal tumor grows less than normally. However, as the first tumor grows usually only when the second grows, this alternating type of growth is not fully realized. It is probable that the second, subcutaneous tumor grows more actively under these conditions because the subcutaneous tissue is a better soil for tumor growth than the peritoneum. Furthermore, we must consider that at the time when the second tumor heated for thirty minutes is inoculated subcutaneously the first, intraperitoneal tumor has hardly started to grow, so that the second, subcutaneous tumor has time to begin growth and become established before the

TABLE 2
ANALYSIS OF TAKES

Retrossions of the Double Inoculations	(1) Tumor Subcutaneous (75 mg., Unheated), (2) Tumor, Subcutaneous			(1) Tumor Subcutaneous (75 mg., Unheated), (2) Tumor, Intraperitoneal		
	(2) 75 mg. Unheated	(2) 75 mg. Heated 25'	(2) 75 mg. Heated 30'	(2) 75 mg. Unheated	(2) 75 mg. Heated 25'	(2) 75 mg. Heated 30'
First and second tumors grew.....	19	21	6	8	3	8
First tumor grew; second did not grow	..	11	7	22	16	24
First tumor grew; second retrogressed..	6	3
First tumor retrogressed; second grew..
First tumor retrogressed; second did not grow	2	3	..	4	4	1
First tumor retrogressed; second retro- gressed	1	16	7	..	1	..
First tumor did not grow; second grew
First tumor did not grow; second did not grow	1	4	4	5	1	5
First tumor did not grow; second retro- gressed	1

full, inhibiting effect of the intraperitoneal tumor develops. When the first tumor is inoculated intraperitoneally and the second tumor subcutaneously, there is no effect of either heated or unheated second tumor on the first tumor, either unheated or heated twenty-five minutes, in a similar manner as a second, subcutaneous tumor has no action on a first, subcutaneous tumor. We have discussed the action of a second tumor on a first tumor heated thirty minutes.

INFLUENCE OF A FIRST, SUBCUTANEOUS TUMOR ON A SECOND, INTRAPERITONEAL TUMOR

When an unheated, therefore an actively growing, subcutaneous tumor has been inoculated first, we find that the second, intraperitoneal tumor is inhibited in its growth. The unheated tumors are

inhibited as much as the heated tumors (the low percentage of growth in the case of the tumor heated for twenty-five minutes, inoculated intraperitoneally may be due to some accidental condition); relatively however, the unheated, intraperitoneal tumors are inhibited more by the first, subcutaneous tumor than are the heated tumors. Thus, while the unheated, intraperitoneal tumor usually grows in 53 per cent and the tumors heated for twenty-five and thirty minutes, in respectively 43 and 34 per cent of the animals, when these tumors are inoculated after a first, subcutaneous tumor they grow respectively in 19, 12, and 21 percent of the animals (Table 1). We have reported the same observation in the case of a combination of first, intraperitoneal and second, subcutaneous tumors. The first, sub-

TABLE 2
ANALYSIS OF TAKES

(1) Tumor Intraperitoneal (75 mg., Unheated), (2) Tumor, Subcutaneous			(1) Tumor (75 mg., Unheated), Subcutaneous (2) Tumor (375 mg., Unheated), Intraperitoneal	(1) Tumor (375 mg., Unheated), Intraperitoneal (2) Tumor (75 mg., Unheated), Subcutaneous	(1) Tumor Heated 25'; (2) Tumor Heated 25'		(1) Tumor Heated 30'; (2) Tumor Heated 30'	
(2) 75 mg. Unheated	(2) 75 mg. Heated 25'	(2) 75 mg. Heated 30'			(1) Subcu- taneous (2) Subcu- taneous	(1) Intra- peritoneal (2) Subcu- taneous	(1) Subcu- taneous (2) Subcu- taneous	(1) Intra- peritoneal (2) Subcu- taneous
14	10	8	12	14	17	16	5	15
15	11	6	8	13	1	9	7	4
..	..	1	3	4	1	1	5	..
..	1	4
4	9	4	6	8	4	8	1	2
..	9	..	1	..	2	5
1	1	..	26
30	14	22	9	16	3	19	..	18
..	1	2	1

cutaneous tumor inhibits the growth of a second, unheated tumor, or intraperitoneal tumor heated for twenty-five minutes, relatively more than it does the growth of similar, subcutaneous tumors, in accordance with the weaker growth of intraperitoneal tumors generally (Table 1).

In all cases, the second, intraperitoneal tumor grows only when the first, subcutaneous tumor grows, and, with the exception of the case about to be discussed, exerts no retarding influence on the first subcutaneous tumor.

Such a retarding influence on the first tumor is noticeable under the following condition: When five pieces (375 mg.) of unheated tumor material are inoculated intraperitoneally, six days after the subcutaneous inoculation of one piece (75 mg.) of unheated tumor

material, we find that, even tho the growth of the intraperitoneal tumor is diminished, it exerts an inhibiting action on the growth of the first, subcutaneous piece. On the other hand, the effect of the subcutaneous tumor on the five pieces is probably less marked than on one piece inoculated intraperitoneally.

We must note especially that even in cases in which the second, intraperitoneal tumor did not grow it exerted an inhibiting influence on the first tumor. Furthermore, there must already be present some immunizing effect on the part of the second tumor at a time even before the second shows any growth. It is evident, however, from the larger percentage of growth of the first tumor that the subcutaneous tumor is more resistant to the immunizing than the second, intraperitoneal tumor. The inhibition of growth of the first tumor must react on the second intraperitoneal tumor and further tend to suppress its growth indirectly.

There are three possibilities of interaction of two such tumors: First, the inoculated animal may be to such a degree naturally resistant that the growth of both tumors is suppressed. Second, a slight degree of natural resistance (not sufficient to inhibit an actively growing tumor) may be added to the inhibiting effect exerted by the second tumor on the first, as well as by the first on the second tumor. As a result of these influences either both tumors, or only the second may be prevented from growth. Third, the combined action of a weak, natural resistance and the inhibiting action of both tumors on each other may not be sufficient to prevent the growth of either the first or the second tumor. We find (Table 2) that all three of these eventualities were realized.

When we compare the influence of a first, subcutaneous tumor on a second, intraperitoneal tumor with the influence of a first, intraperitoneal tumor on a second, subcutaneous tumor, we find that relatively (comparing the percentage of growth of each tumor when inoculated second with the normal percentage of growth in case of a single inoculation) a second, intraperitoneal tumor is inhibited to a smaller extent by the first, subcutaneous tumor than a second, subcutaneous tumor by a first, intraperitoneal tumor. It is therefore evident that the inhibiting influence exerted by an intraperitoneal tumor is greater than that of a subcutaneous tumor, and that, even tho a second, intraperitoneal tumor grows in a smaller percentage

than a second, subcutaneous tumor, a second, subcutaneous tumor is relatively more inhibited in its growth than a second, intraperitoneal tumor (Table 1).

Krauss, Ranzi, and Ehrlich,² who found in their experiments that an intraperitoneal tumor was a more actively growing one than a subcutaneous tumor, came to the conclusion that the intraperitoneal tumor immunized more actively against a second inoculation than did a subcutaneous tumor. Our results agree to some extent with those of these investigators, in as far as they found that intraperitoneal tumors immunized more actively than did subcutaneous tumors. Since they had found in their experiments that the intraperitoneal tumor was the more actively growing, their conclusion was in agreement with the results which had been obtained by Loeb¹ and others to the effect that an actively growing tumor has greater immunizing powers than a less virulent tumor. We have, however, shown in our experiments that even using a tumor which, when inoculated intraperitoneally, grows less actively than when inoculated subcutaneously, we were still able to demonstrate the greater immunizing effect of the weaker, intraperitoneal tumor. Therefore, it appears that the greater immunizing power of the intraperitoneal tumor is due to the location of the tumor and not because it is a more actively growing tumor.

Krauss, Ranzi, and Ehrlich state also that an intraperitoneal tumor caused general immunity, while a subcutaneous tumor caused only local immunity against a subcutaneous tumor but not against an intraperitoneal tumor. We find that both intraperitoneal and subcutaneous tumors exert a general immunity, but that the immunizing effect of the intraperitoneal tumor is stronger. The fact that they used a different tumor from that which we used and that they did not vary the virulence of their tumor may account for the differences in results.

We have also observed the rate of growth as influenced by the double inoculations. Since it is difficult to determine definitely the size of intraperitoneal tumors by palpation and owing to the relatively small number of mice examined in certain cases, we hesitate to draw any definite conclusions from these observations. If, however, in the future opportunity arises to extend these observations we hope to publish these results. We may state that such observations as have been made do not change or affect the conclusions drawn from the observations made on the percentage of takes.

SUMMARY

The number of takes after intraperitoneal inoculation is smaller than after subcutaneous inoculation. After inoculations of unheated (virulent) as well as of heated tumors (less virulent), the number of takes is approximately 40 percent less in the case of intraperitoneal inoculation as compared to the number of growing, subcutaneous tumors treated in the same manner before inoculation.

Notwithstanding the diminished number of takes, the immunizing effect of intraperitoneal tumors is markedly greater than that of subcutaneous tumors, altho the subcutaneous tumors have likewise a decided inhibiting effect on the growth of intraperitoneal tumors.

In a similar manner as the subcutaneous tumor with markedly diminished virulence may exert a favorable influence on another subcutaneous tumor of diminished virulence, a subcutaneous and intraperitoneal tumor may under certain conditions exert a favorable influence on each other as shown by the number of successful inoculations.

The immunizing effect of a first unheated, intraperitoneal tumor on a second, subcutaneous tumor is somewhat greater if the second, subcutaneous tumor had been heated, but even so the inhibiting effect on a more actively growing unheated, second subcutaneous tumor is as evident as on a heated, second tumor. Second, heated, subcutaneous tumors grow almost as well as second, unheated, subcutaneous tumors when in combination with a first intraperitoneal tumor.

If, on the other hand, the first, intraperitoneal tumor is heated it gradually loses its inhibiting power and even a favorable action of the first, heated, intraperitoneal tumor on a second, subcutaneous tumor becomes the more apparent the more the second tumor has been heated.

If the second subcutaneous tumor has been heated only twenty-five minutes the favoring action of a first, intraperitoneal tumor, heated for thirty minutes, is not marked. But when a second, subcutaneous tumor, heated for thirty minutes, follows an intraperitoneal tumor heated for thirty minutes, the second tumor is favored while the first is slightly inhibited. When both tumors are subcutaneous, the first tumor has the advantage of time and thus grows better than normal, while the second is now inhibited. Therefore we observe here similar phenomena as those described in our first communication.¹

A second, subcutaneous tumor has usually no inhibiting action on any first tumor, whether it is subcutaneous or intraperitoneal, except-

ing possibly the inhibiting action on the part of a second tumor in the case mentioned above, of the two tumors heated for thirty minutes.

A first unheated, subcutaneous tumor inhibits a second, intraperitoneal tumor markedly; it has, however, slightly less inhibiting power on a second tumor after 375 mg. have been inoculated intraperitoneally second than after inoculation of 75 mg.

A first unheated, subcutaneous tumor has a relatively smaller inhibiting effect on a second, heated (twenty-five or thirty minutes), intraperitoneal tumor than on a second, unheated, intraperitoneal tumor. If both tumors were subcutaneous, the inhibiting effect on the second, heated tumor would be greater, while if the second were a unheated subcutaneous tumor the inhibiting effect of a first, unheated subcutaneous tumor would be very slight.

If a large quantity of a second, unheated, intraperitoneal tumor is inoculated (375 mg.) it may even inhibit a first, unheated, subcutaneous tumor. Intraperitoneal inoculation of smaller quantities has no such effect. A first intraperitoneal tumor inhibits a second subcutaneous tumor even in cases in which itself does not grow.

Therefore, we may conclude that both intraperitoneal as well as subcutaneous tumors have a mutual inhibiting action on each other if they are virulent or moderately virulent, but that an intraperitoneal tumor has a relatively stronger inhibiting power than a subcutaneous tumor. The greater inhibiting action of an intraperitoneal tumor on a subcutaneous tumor appears when compared with the action of a first, subcutaneous tumor on a second, subcutaneous tumor, as well as on a second, intraperitoneal tumor. A second intraperitoneal tumor is relatively more strongly inhibited by a first, subcutaneous tumor than is a second, subcutaneous tumor.

EXPERIMENTAL STUDY OF THE DISTRIBUTION AND HABITAT OF THE TETANUS BACILLUS *

WILLIS NOBLE

(From the Sheffield Scientific School of Yale University, New Haven, Connecticut.)

In this work an attempt has been made to determine the importance of some of the herbivorous animals as carriers or distributors of tetanus spores. With this object in view, the feces of many animals have been examined and the following points have been emphasized: How many of the animals harbor the organism in the intestines? To what extent are tetanus spores present in the feces of such animals? Do such animals become permanent carriers, or do they eventually rid themselves of the organism? Can the tetanus bacillus multiply in the animal intestine?

HISTORICAL REVIEW

The tetanus bacillus appears to be of almost universal distribution in nature. Lortet¹ found it in the mud dredged from the bottom of Lake Geneva as well as in the waters of the Dead Sea. Ledantec² reports of having found it in the poisoned arrows of the natives of the New Hebrides, who obtained it by smearing their arrow heads with the material found in the burrows of large crabs. Peyraud³ has found it in 50 percent of the samples of hay dust examined by him, and Ringeling⁴ reports its presence in the bilge water of ships. The investigations of many other workers show it to be frequently present on wearing apparel,⁵ upon the skin of man and animals, in gun wads, and in different soils. Nicolaier⁶ found it in twelve out of eighteen samples of soil collected about Göttingen, and of one hundred and ninety-two specimens from Leipzig, Berlin, and Wiesbaden, eighty-one showed it when inoculated into mice. Bossano⁷ in Marseilles tested the soils of thirty-eight cities in different parts of the world, and samples from only twelve of the cities were free from it.

Generally, when the bacillus has been found in soils, it has been garden soil, especially soil fertilized with animal feces, or dust and dirt from streets. In his own work, Nicolaier observed that the samples containing the bacillus came from gardens, court-yards, streets, or riding halls, but that unfertilized soil did not contain it. Bissérie⁸ likewise found earth contaminated with animal feces to be highly infected with tetanus spores, while forest soils were not.

* Received for publication November 23, 1914.

1. *Centralbl. f. Bacteriol.*, 1891, 9, p. 709; *Ibid.*, 10, p. 567.

2. *Ann. de l'Inst. Pasteur*, 1890, 11, p. 716.

3. *Centralbl. f. Bakteriologie*, 1891, 9, p. 17.

4. Quoted in Kolle and Wassermann: *Handbuch der pathogenen Mikroorganismen*.

5. *Centralbl. f. Bakteriologie*, 1908, 41, p. 390.

6. *Inaug. Diss.*, Göttingen, 1885; *abs.*, Baumgarten's *Jahresbericht*, 1886, 2, p. 270.

7. Quoted in Kolle and Wassermann: *Handbuch der pathogenen Mikroorganismen*.

8. *Ibid.*

Sormani,⁹ in 1899, found tetanus bacilli in the freshly deposited feces of many animals, and Chicoli Nicola¹⁰ found them to be especially abundant in horse feces. Sormani¹¹ and others further showed that tetanus is not produced in animals by feeding the organism, but that the feces of such animals will subsequently contain it. Pizzini¹² found the bacillus in 5 percent of the human feces examined. His figures are striking, for they show that 30 percent of the men working about horses and stables have tetanus bacilli in their feces, while only 2.2 percent of men engaged in other occupations have them.

The frequent occurrence of the bacilli in the animal intestine is highly suggestive. It is readily conceivable, when we consider the wide distribution of the spores in the soil, hay-dust, grain, etc., that their normal mode of entry into the digestive tract is by ingestion with food, or through breathing and swallowing infected air. That this may occur in the case of the human intestine has been shown by Pizzini,¹² and more recently by Freund¹³ in the report of a case of puerperal tetanus, in which the patient was undoubtedly infected through her own feces. Whether the spores that are swallowed remain inactive, provided they do not obtain a lodgement in a wound or lesion of the digestive tract, and are quickly eliminated in the feces, or whether they effect a permanent, or even a temporary, foothold in the intestine is a question which has led to much controversy. Thus, Sormani,¹⁴ after finding the bacillus in the feces of many animals, advanced his fecal theory to explain the universal distribution of the organism. He believes that passage through the digestive tract is a necessary phase in the life activities of the bacillus, that in the intestine it multiplies and renews its virulence, and that its spores are then passed out in the feces and so spread over the surface of the ground. He believes that it cannot multiply in the soil because of unfavorable meteorological conditions, such as light and the lack of moisture, that it gradually loses its virulence there, and that only the extreme resistive powers of its spores enable it to survive. On the other hand, with the absence of oxygen, with the favorable temperature and rich food supply in the animal intestine, conditions should be most favorable for its growth.

The other side of this controversy has been taken by Vincent¹⁵ with his "telluric theory." While admitting the presence of the tetanus bacillus in the intestines of many of the herbivora, he doubts that it multiplies there since the feces of all of the animals he examined did not show it. He has fed rabbits small amounts (1 c.c.) of spore-containing cultures and killed the rabbits at the end of twelve hours. He claimed that microscopic examinations and animal inoculations from cultures made along different parts of the alimentary canal showed no increase in the number of tetanus bacilli. In another series of experiments, he reports feeding tetanus spores to rabbits and guinea-pigs in which the pylorus was ligated, and he finds that "the number of spores in the stomach is greatly diminished by the end of two hours." He finally shows that tetanus bacilli will not grow readily *in vitro* when planted in pancreatic extract, or in the extract from the intestinal wall; furthermore, "bile shows an antiseptic action" on them. Accordingly, he believes that they must grow in decomposing organic matter, such as the rotting straw and manure in the stable, where they are protected from the light, and where moisture and the

9. *Centralbl. f. Bakteriöl.*, 1890, 7, p. 250.

10. *Ibid.*, 1891, 9, p. 18.

11. *Ibid.*, 1891, 10, p. 421.

12. *Baumgarten's Jahresbericht*, 1894, 14, p. 236.

13. *Ztschr. f. Geburtsh. u. Gynäk.*, 1912, 72, p. 97.

14. *Centralbl. f. Bakteriöl.*, 1892, 12, p. 609.

15. *Comp. rend. Soc. de biol.*, 1908, 65, p. 12.

heat of decomposition, together with the presence of many aerobic bacteria which absorb oxygen, offer favorable conditions.

His evidence is not entirely conclusive. That he could not detect any increase in numbers twelve hours after the feeding of spores to a rabbit does not seem strange if we consider, first, the difficulty of making even an approximate numerical comparison between the spores fed and those recovered from the intestine, and, second, that even under the most favorable conditions of artificial cultivation we can never detect growth of tetanus bacilli as early as twelve hours after planting, and only exceptionally can we do so by the end of twenty-four hours. That the number of spores in the stomach with the pylorus ligated should be greatly reduced two hours after feeding does not seem at all peculiar in view of the abnormal conditions and the long exposure to the acidity of the gastric juice. He advances no direct experimental evidence that the tetanus bacilli multiply in decomposing organic matter, and in opposition to his theory that they do so is the evidence of Bombicci¹⁶ and of Von Esmark¹⁷ that these bacilli quickly disappear from putrefying mixtures, and that their spores can be demonstrated in the surrounding material for only a short time thereafter.

The conflicting evidence thus presented by Vincent and Sormani as to the manner of growth and multiplication has made it desirable to undertake the work herein reported in the hope that different technic and methods of cultivation might throw further light upon this subject.

METHODS OF CULTIVATION AND EXAMINATIONS

As a preliminary to the actual experimental work, many methods were tested for the purpose of finding one for cultivating tetanus bacilli which would be both reliable and simple. Smith's¹⁸ tissue method for cultivating anaerobes, as modified by Tarozzi,¹⁹ was eventually selected as one well suited to the requirements of this work. The medium was prepared as follows:

Test tubes were filled with the ordinary Liebig's beef extract nutrient broth and sterilized in the usual way. The tissues of young and healthy white rats, guinea-pigs, rabbits, and cats were used. The animals were chloroformed, thoroughly washed with a 1:1,000 solution of mercuric chlorid, the skin stripped from the abdomen, and the latter opened with sterile instruments. The liver and kidneys were removed from the peritoneal cavity, with all aseptic precautions, to large covered Petri dishes prepared to receive them, and care was taken to avoid the gall-bladder while removing the liver. Small pieces (approximately 1 gm. of tissue for each 10 c.c. of broth) were then cut off and dropped into the broth tubes. These were then incubated for two days, when they were ready for use. By this method, with reasonable care, there is little danger of contamination, and the period of preliminary incubation is of advantage in that tubes which may have been contaminated will show growth and may be rejected.

16. Baumgarten's Jahresbericht, 1891, 7, p. 220.

17. Ztschr. f. Hyg. u. Infektionskrankh., 1889, 7, p. 1.

18. Centralbl. f. Bakteriöl., 1890, 7, p. 502; Jour. Med. Research, 1906, 14, p. 193.

19. Centralbl. f. Bakteriöl., 1905, 38, p. 619

Routine Examinations.—The feces of sixty-one horses, twenty-one cows, and one guinea-pig were examined for tetanus spores.

The feces were collected immediately after being passed and brought to the laboratory. Material from the center of the fecal masses in the cases of the horses and cows was weighed out in sterile Petri dishes. Examinations were made of both unheated and heated feces. The unheated feces, after being weighed out in 10 gm. and 1 gm. lots, were planted directly in Smith's¹⁸ tissue broth in the proportion of an approximate 10 percent suspension and then incubated at 37 C. The feces which were to be examined in a heated condition were first planted in tubes of plain broth, in a 20 percent suspension, and the tubes then placed in the water bath at 80 C. for ten minutes. After being cooled to 40 C. an equal volume of tissue broth was added, and the cultures then incubated at 37 C. This procedure was adopted to avoid heating the tissue broth, since heat destroys the efficacy of the tissue to support the growth of anaerobic bacteria. At the expiration of from six to ten days, smears were made from both unheated and heated cultures, stained by Gram's method, and examined for tetanus-like, terminal, spore-bearing bacilli. From the cultures showing such forms, young white rats were inoculated with 1 c.c., and from those rats dying with typical symptoms of tetanus the bacillus was recovered by dropping bits of tissue or of pus from the site of inoculation into tissue broth tubes, or by scraping the wound with a platinum loop and inoculating tissue broth tubes. Cultures thus made were grown for one week and then tested by inoculating them into white mice.

Of the sixty-one horses examined, eleven, or 18 percent, showed tetanus bacilli in their feces. No tetanus bacilli were found in the feces of the cows. Park²⁰ states that 15 percent of horses and calves in the vicinity of New York City harbor tetanus bacilli in their intestines. Sanchez-Toledo and Veillon²¹ obtained tetanus bacilli from the feces of four horses out of six examined, and of one cow, the only one tested. It would seem that our figures compare favorably with those given by Park²⁰ for horses. Sanchez-Toledo and Veillon²¹ have examined but few animals, and hence it is unfair to compare our results with theirs.

Our examinations of cow feces were made during the winter months at a time when the animals were confined in a barn (all the animals belonged to one dairy), and when their diet was largely of ensilage, a highly fermented food and therefore one not likely to contain tetanus spores. Some of our results seem to indicate that the tetanus bacillus, when planted in the intestines of certain individual animals, will remain there but a short time, being eventually eliminated. This may explain our failure to find the organism in cow feces.

20. Pathogenic Bacteria and Protozoa, 1910, p. 232.

21. Centralbl. f. Bakteriöl., 1891, 9, p. 18.

The feces of one guinea-pig were examined for tetanus bacilli, and rats and mice that were inoculated died of tetanus in forty-eight hours. Sanfelice²² has reported the recovery of tetanus spores in the feces of seven normal guinea-pigs out of twenty-three examined by him.

Quantitative Examinations.—To determine to what extent the tetanus bacillus is present in the feces of animals which have been shown to harbor it within their alimentary canals, quantitative bacteriological examinations of the feces were made. For this purpose "Nora" and "Lady," two horses known to harbor the organism, were used. These quantitative determinations were made as follows:

One gram of horse feces from the interior of a fecal ball was planted in 10 c.c. of plain broth. This suspension was placed in an automatic shaker for one hour to obtain a uniform emulsion. Dilutions of 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000 were then made successively by transferring 1 c.c. of this fecal emulsion into 9 c.c. of tissue broth, and so on. The experiment was done in duplicate; one of the tubes containing the initial suspension of feces was heated to 80 C. for ten minutes before the dilutions in the tissue broth were made. After incubation of the culture tubes for six days, inoculations were made into young white rats. The results are recorded in Table 1.

TABLE 1
SHOWING RESULTS OF QUANTITATIVE EXAMINATION OF FECES OF TWO HORSES CARRYING
TETANUS BACILLI

February 10—"Lady"			February 25—"Nora"	
Amount, in Gm., of Feces Tested	Cultures Tested Without Prelim- inary Heating	Cultures Heated 80 C. for 10 Minutes Before Inoculation	Cultures Tested Without Prelim- inary Heating	Cultures Heated 80 C. for 10 Minutes Before Inoculation
1 0.1 0.01	+	+	+	+
	0	0	0	0

From these figures it seems either that tetanus bacilli are not present in large numbers in the intestine of the horse or our present-day methods of making quantitative bacteriological determinations, at least in the case of anaerobes, are not sufficiently accurate and reliable to detect an isolated spore or a small group of organisms.

FEEDING EXPERIMENTS

Two sets of experiments have been carried out for the purpose of determining whether or not an animal the intestines of which have once been infected with tetanus spores becomes a permanent carrier.

Guinea-pigs have been given single feedings of relatively large amounts of cultures containing tetanus spores, and their feces examined subsequently at frequent intervals.

These experiments were divided into two periods: A fore-period, during which examinations of the feces were made to determine if the animal's intestines were already infected with tetanus spores; and an experimental period, during which the animal received one feeding of tetanus spores. After this frequent examinations of the feces were made to determine for how long a time the spores continued to be voided.

Two full-grown guinea-pigs were put into metabolism cages provided with bottoms of coarse wire through which the feces dropped as quickly as passed. It was necessary to do this, as the pigs were found to be coprophagous because of their restricted diet. The feces were collected while fresh and planted in tissue broth tubes. Inoculations into young white rats were made after incubating the cultures for from one week to ten days.

The fore-period of the experiment lasted for eleven days. Three examinations of the feces were made during this time. All failed to show tetanus bacilli on animal inoculation. At the end of the fore-period, Guinea-pig 1 was given one feeding of 5 c.c. of a ten-day broth culture of tetanus spores. Guinea-pig 2 was used as a control to indicate whether or not after this single feeding of tetanus spores to Guinea-pig 1 there was any subsequent ingestion of tetanus spores with the food. The diet was restricted, consisting of bread and lettuce carefully washed in sterile water. Table 2 following shows the results for the experimental period.

TABLE 2
RESULTS OF EXPERIMENTAL PERIOD ON GUINEA-PIGS 1 AND 2

Guinea-Pig 1 (Experimental Animal)				Guinea-Pig 2 (Control Animal)	
Date	Weight, in Mg., of Feces Examined	Rat Inoculation	Mouse Inoculated with Culture Recovered from Rat Dying of Tetanus	Weight, in Mg., of Feces Examined	Rat Inoculation
March 12	300	0	..	300	0
March 13	400	0	..	300	0
March 15	600	0	..	400	0
March 18	1,000	+	+	1,000	0
March 20	1,500	0	..	500	0
March 24 *					

* Pig died but not of tetanus

The sign + indicates that inoculated animal died of tetanus; 0 indicates absence of tetanus reaction.

Just before Guinea-pig 1 was fed the tetanus culture, a capsule of powdered carmin was administered. The carmin was completely eliminated in the feces in twenty-four hours, but no tetanus spores could be demonstrated until the seventh day after feeding. That their appearance in sufficient numbers to indicate their presence in

the feces at that time was due to some second accidental ingestion of spores, possibly with the food, is improbable since the control pig, kept under the same conditions and fed with the same food, should also have shown them in his feces had this happened. This was not the case, and it is more reasonable to suppose that the spores were being continually eliminated during these seven days, but in numbers too few to respond to cultivation.

On March 18, the day on which the tetanus spores appeared in the feces of Guinea-pig 1, a quantitative examination for the spores was made. One gram of feces was thoroughly shaken in a machine in 10 c.c. of broth. Dilutions containing the amounts of fecal material shown in Table 3 were made from this and after cultivation for one week were tested on white rats in the usual way.

TABLE 3
SHOWING RESULTS OF QUANTITATIVE EXAMINATION FOR TETANUS SPORES IN FECES OF GUINEA-PIG 1

Amount, in Mg., of Feces Tested	Result of Rat Inoculation	Result of Inoculation of Mouse with Cultures Recovered from Rat Dying of Tetanus
1,000	+	+
100	+	+
10	+	—
1	0	

The sign + indicates that the inoculated animal died of tetanus; — the failure to recover the tetanus bacillus from the rat dying of tetanus; 0 indicates absence of tetanus reaction.

In order to secure additional data, this entire feeding experiment was repeated on two normal guinea-pigs. As in the first case, the experiment was preceded by a fore-period of eleven days, during which the feces were collected and tested four times for tetanus. These four tests were all negative. The methods of examination and all other details were the same as before, and so only the tabulated results are given (Table 4).

In this last feeding experiment it will be noted that 10 c.c. of a spore-containing culture were given, whereas in the first case 5 c.c. were fed. The results are radically different, for in the second case the spores are eliminated continuously from the second day after feeding to the sixteenth, after which they disappear, while in the first case they appear once, on the seventh day after feeding. A possible explanation of this is that in the first case spores may have been

TABLE 4
RESULTS OF EXPERIMENTAL PERIOD OF TWENTY-FIVE DAYS

Guinea-Pig 3 (Experimental Animal)			Guinea-Pig 4 (Control Animal)	
Fed 10 c.c. of a Twelve-Day Broth Culture of Tetanus Spores				
Date	Weight, in Mgs., of Feces Examined	Result of Rat Inoculation	Weight, in Mgs., of Feces Examined	Result of Rat Inoculation
April 8	500	+	600	0
April 10	500	+	400	0
April 11	550	+	300	0
April 12	300	+	450	0
April 23	500	+	400	0
April 30	400	0	250	0
May 3	500	0	400	0

The sign + indicates that the inoculated animal died of tetanus; — the failure to reaction. The feces of the control pig contained tetanus spores at no time during the experiment.

given off continuously in the feces in numbers too few to be detected by our methods, but if multiplication took place in the intestine it is conceivable that by the seventh day after feeding, sufficient numbers had begun to appear in the feces to respond to culture methods, as we have already suggested. In the second case, however, the initial dose of spores was sufficiently large to enable them to pass through in the feces from the very beginning. Unfortunately, Guinea-pig 1 died before further tests could be made. It is interesting to compare these results with those obtained by Sormani,²³ who found that tetanus spores persisted in the feces of a dog for sixteen days after feeding.

Two horses, "Nora" and "Lady," the feces of which had been shown to contain tetanus spores, were examined for the organism at different intervals for a period of six months. From the same stable two other horses, "Beauty" and "Floradora," which previously had not shown tetanus spores in their feces, were used as controls to show whether or not all the horses were subjected to fresh infections of their alimentary canals with tetanus spores during the period of observation. One gram of feces was tested and cultures were made on Smith's tissue broth and incubated for from six to ten days. The results of the experiment are given in Table 5.

23. Centralbl. f. Bakteriöl., 1891, 9, p. 421.

TABLE 5
RESULTS OF EXAMINATION OF TWO HORSES FROM WHICH TETANUS SPORES HAD BEEN
OBTAINED BEFORE AND OF TWO HORSES WHICH IN PREVIOUS TESTS GAVE
NEGATIVE RESULTS

Date	Experimental Horses		Control Horses	
	"Nora"	"Lady"	"Beauty"	"Floradora"
January 20	+	+	0	0
January 27	+	+	0	0
February 3	+	+	0	0
February 10	+	0	0	0
February 20	+	Not tested	0	0
March 3	+	0	0	0
April 30	+	0	0	0
June 13	0	0	0	0
June 23	0	0	0	0
June 26	0	0	0	0
June 28	0	0	0	0
July 1	0	0	0	0
July 24	0	0	0	0

The sign + indicates that the rat inoculated died of tetanus. Dilutions showed that the feces of "Nora" contained tetanus spores in 0.1 gm., but not higher dilutions.

The results for "Nora" are extremely interesting, for they show that individual animals may harbor tetanus bacilli in their digestive tracts for many months following an initial infection. "Lady," on the other hand, appears to have eliminated the organism completely within fourteen days after the beginning of observation.

DISCUSSION OF RESULTS

If we refer to the results of the experiments in which tetanus spores were fed to guinea-pigs, it will be seen that one of the pigs so fed harbored spores for a period of sixteen days, after which the spores disappeared from the feces. Another pig showed spores on the seventh day after feeding, but unfortunately the experiment ended at that time because of the death of the animal. Tests on the two horses "Nora" and "Lady" show that the spores disappeared from the feces of "Lady" fourteen days after the beginning of the observations, while "Nora" harbored them continuously for four months, altho, here too, the organism eventually disappeared. The horses used as controls during this period of four months showed that no new infection at least of sufficient magnitude to be detected, took place. Quantitative tests have shown that tetanus spores may be found in the feces of animals carrying them in such amounts of feces as 0.1-0.01 gm., but not in smaller quantities. It is possible that our present-day methods of cultivation are responsible for failure

to detect the spores in smaller amounts of feces, for to insure growth under artificial conditions it is generally necessary to transplant relatively large amounts of the tetanus cultures. It must be remembered also that the tetanus bacillus grows slowly, even under the most favorable conditions. Considering these facts, it seems inconceivable that tetanus spores can remain in the intestine even for sixteen days, being constantly voided in the feces during that time, unless they maintain a foothold there by multiplication. Surely this conclusion is strengthened by the observations on "Nora" which has been shown to harbor the organism, not for sixteen days only, but for four months. Why this one animal should carry the organism for such a long period, while the others were free from it, or had rid themselves of it so soon, is not clear, but the explanation is probably to be found in some difference in the nature of the intestinal contents, or the intestinal flora. The phenomenon of the human typhoid or cholera carrier is well known. It seems that we are dealing with a "tetanus carrier" in the case of "Nora" in so far as the distribution of tetanus spores is concerned. What the percentage of such "tetanus carriers" is to normal animals is a problem which remains to be investigated; but granting the existence of "tetanus carriers" the universal distribution of tetanus spores is at once explained.

SUMMARY

The tetanus bacillus appears in the intestines of many normal animals, especially of the herbivora, but apparently it seems impossible, with the methods at our disposal, to detect it there unless it is present in relatively large numbers.

Experimental evidence shows that the tetanus bacillus may multiply in the intestines of such animals.

The intestines, or rather the intestinal contents of certain individual animals, seem to offer especially favorable conditions for the growth of the tetanus bacillus; such animals are "tetanus carriers" comparable, in regard to the distribution of the organism, with typhoid or cholera carriers among human beings.

The presence of tetanus spores in soils, street dust, fresh vegetables, and on clothing and the skin is undoubtedly due to fecal contamination.

SYPHILITIC LEPTOMENINGITIS *

E. R. LECOUNT AND KAETHE DEWEY

(From the Pathological Laboratory of Rush Medical College and the Otho S. A. Sprague Memorial Institute, Chicago, Illinois)†

WITH PLATES 1 TO 11

The only unanimity among the writers on syphilis of the central nervous system, with respect to the various forms of syphilis of the meninges, is the recognition by all of a gummatous meningitis. The spreading exudative leptomeningitis, generally accepted as the commonest form of syphilis of the meninges, has a number of names, for example, diffuse gummatous meningitis (Oppenheim), fibrohyperplastic syphilitic meningitis (Nonne), and "m ningite scl rogommeux" (S zary). The terms, disseminated, diffuse, circumscribed, and mixed, are used with reference to distribution of the lesions and need no comment. In short, syphilis in this part of the body is characterized, as it is usually elsewhere, by focal lesions, the gummas. Various names have been applied to the disease because of the variations in size and location of the gummas and the extent to which they may or may not be accompanied by inflammation, spreading out from their periphery and perhaps coalescing to form more or less of a fibrinocellular exudate, which varies in its character according to the degree of acuteness and of healing.

REVIEW OF THE LITERATURE

S zary¹ classifies syphilitic meningitis according to the principles which govern the general pathology of syphilis: Secondary syphilitic meningitis, characterized by diffuseness and generalization of the process with a tendency to resorption; tertiary meningitis, characterized by circumscribed lesions with a tendency to necrosis and fibrous thickening; "residual," "metasyphilitic," or "parasyphilitic" meningitis, which accompanies dementia paralytica and tabes. According to S zary, the latter form of meningitis is not syphilitic.

In text-books we generally find a description of the basilar exudative form. Oppenheim² states: "I do not hesitate to call diffuse basilar syphilitic meningitis the most common form of syphilitic affections of the brain. . . . The base looks as if some solidifying fluid, like paraffin, has been poured over it. The entire base, or chiefly the interpeduncular space and the region of the optic chiasma, are thus involved. The mass penetrates into all fissures and recesses, so that the basal structures are more or less hidden by it. Also, the roots of

* Received for publication November 25, 1914.

† Aided by a grant from the Committee on Scientific Research of the American Medical

1. Jour. de m d., Paris, 1913, 7, p. 201.

2. Berl. klin. Wchnschr., 1889, 26, p. 1033.

the cranial nerves are imbedded in this substance. The new formation is gelatinous, or slightly elastic in consistency. In some places, it forms a tough plaque of connective tissue which is intimately adherent to the basal portions of the brain."

Meningitis of the convexity is considered as of much rarer occurrence. "The same process of a gummatous meningitis, sometimes circumscribed, sometimes spreading over a considerable area, may develop on the convexity of the brain, tho not so often as at its base. In the former case, it penetrates more or less deeply into the tissue of the brain and thus affects the functions. It has also been observed that the same process may involve the base and the convexity of the brain simultaneously" (Oppenheim). In fact, reports often mention an occasional involvement of the membranes of the convex surface of the brain or a milky opacity of the vessels in the fossa Sylvii, but it is frequently represented as an extension from the base.

Besides nomenclatures, there are other phases of the disease about which the authors differ. Oppenheim writes as follows: "I want to emphasize the fact that syphilis is rarely limited to the spinal cord; in the majority of cases it is of the type of a cerebrospinal involvement . . . so that I may designate diffuse syphilitic cerebrospinal meningitis as the most frequent form of syphilitic diseases of the central nervous system." Nonne's observations on this point, however, are: "In my material, I have never seen this extensive and high grade gummatous involvement of the spinal cord and base of the brain which plays such a great rôle in the literature, and I may state here that all the experienced specialists, of whom I have made inquiries concerning this, have convinced me that these 'classical cases of a gummatous cerebrospinal meningitis' must be very much rarer now."

Recently, a form of syphilitic meningitis, which is not a new type but which has unusual and interesting features, has been discussed under the heading of "nodular" syphilitic meningitis, or syphilitic meningitis with "nodule formation." Nodule means here the small nodule ("Knötchen") which macroscopically does not differ grossly from the tubercle and which for this reason has given rise to difficulties not solely of nomenclature, but also, in some instances at least, of interpretation of the disease. The larger nodule ("Knoten") has such distinctive features that differentiation between it and the tubercle does not really present any difficulty. Baumgarten has demonstrated this very clearly in an interesting report of syphilitic meningitis in a man who also had well-defined pulmonary tuberculosis. There were miliary nodules ("Knötchen") in the lung as well as nodes in the meninges, but minute nodules could not be detected in the latter.

Regarding Baumgarten's report Nonne⁴ states: "I could not find a similar case in the literature; hence this degree of multiplicity (of gummas) is, at all events, a very rare one." In the brain Baumgarten studied, the lesions were located on the base on the midbrain, the circulus Willisi, the left half of the pons, and the adjoining portions of the cerebellum and medulla oblongata, and isolated nodes were found in various parts of the basal arachnoid of the cerebral and cerebellar hemispheres.*

This nodular ("knötchenförmige") syphilitic meningitis seems to be a rare variety of the disease. Dürck's⁵ report (in 1908) of five cases of this type, which aroused a great deal of discussion and controversy, was followed by reports by

* The numbers of lesions in Brain 32 of our series considerably exceed those reported by Baumgarten.

3. Virchows Arch. f. path. Anat., 1881, 86, p. 179.

4. Syphilis und Nervensystem, 1909, p. 38.

5. Verhandl. d. deutsch. path. Gesellsch., 1908, 12, p. 211.

Versé of one case (1908); by Albrecht of two cases (1908); by Beitzke⁶ of three cases (1911), who stated that small nodules in syphilitic meningitis had been mentioned in the literature before Dürck, but that their occurrence had been overlooked or forgotten; and by Sugi⁷ of one case (1912). The small nodules observed by Dürck were mostly on the base, but also along the vessels in the fossa Sylvii, and, in two cases of hereditary syphilis, they were also on portions of the convexity. In Beitzke's cases, the nodules were on the base and in the fossa Sylvii in two cases, but in the third case they were entirely on the convexity. The brain which Sugi described had nodules in some places on the base and medulla. The size of the nodules was variable: in Dürck's cases they were the size of a millet seed or somewhat larger; in Beitzke's, they were the size of a poppy seed in one, submiliary in another, and unequal in size in the third; in Versé's case they were miliary; and in Sugi's, the size of a pin-head. The number of the nodules also varied: in three of Dürck's cases, in one of Beitzke's cases and apparently also in Versé's case they were numerous, while in Sugi's case they were rather scanty.

Microscopically, there are three main sorts of alteration: the focal lesions; the more widely spread, chronic, hyperplastic inflammation; and the vascular changes. Very frequently, there is a combination of these. A combination of the multiplicity of lesions, the evidences of old and new processes, the appearance and disappearance of conditions, degeneration, regeneration, and absorption, is the pronounced microscopic feature. Nonne⁸ points out that in the leptomeninges we "hardly ever miss the combination of the simple hyperplastic form of the acute and chronic inflammation with gummas, more recent processes side by side with later stages, all of which demonstrates so well the chronicity and the tendency peculiar to syphilitic processes to reappear in new crops. . . . The less the inflammatory congestive element prevails, the more evident are the specific neoplastic formations, which, on the other hand, are less pronounced, the more acute the inflammation is, because more time is needed for productive activity." Where the gummatous form is pronounced, the normal configuration of the pia-arachnoid is more or less effaced. Poorly stained areas are filled with degenerated cells and amorphous masses, and giant-cells may be found here and there. Vessels are altered, obliterated, or entirely reorganized, so that remnants of elastic fibers may be all that denotes the vessels' former location. Hyperplasia of the connective tissue is nearly always present; the thickening may be considerable and involve all the trabeculae, longitudinal and oblique. Between these strands there are foci of round-cell infiltration, small or extensive, within the pia along the surface of the brain, or around the vessels, or enclosed in the meshes of the arachnoid membrane and of the subarachnoid space. A close connection with the vessels is generally in evidence; the lymphocytes frequently occupy the adventitial spaces and may form more or less regular coats around the vessels. The immediate neighborhood of vessels is also the place of predilection for small gummas. These may be simply dense, round accumulations of lymphocytes or they may have epithelioid cells and may show within their centers all stages of caseation from beginning karyorrhexis to complete necrosis. Around the centers are radially arranged, epithelioid cells. Giant-cells may be absent or present, scanty or abundant.

Stursberg⁸ describes a brain of which the pia was normal macroscopically, as were also the vessels at the base and the arteriae fossae Sylvii, but, microscopically, a very extensive involvement of almost the entire pia was found.

6. Virchows Arch. f. path. Anat., 1911, 204, p. 453.

7. Wien. klin. Wchnschr., 1912, 25, p. 1827.

8. Deutsch. Ztschr. f. Nervenhe., 1910, 39, p. 459.

The processes were chiefly of an inflammatory nature and consisted of diffuse round-cell infiltration of varying degrees continuing into the nerve tissue as a perivascularitis. Besides these recent processes, there were here and there considerable thickenings of the connective tissue. There were no gummas. The pial arteries were only slightly involved, but the veins were thickened and infiltrated with round cells. Cases of this sort, a diffuse infiltration without gummas, are also described by Möller⁹ and Finkelnburg.¹⁰ Dürk⁵ describes peculiar, large cells, chromataphores, which occur normally in the leptomeninges, but are sometimes greatly increased in syphilis. They often have long and stellate processes and are filled with fine granules of yellowish brown pigment.

The giant-cells, not infrequently formed in gummatous meningitis, have been the subject of discussion. Baumgarten³ considers giant-cells in a granulation tissue as indicative of tuberculosis, but there has been so much convincing evidence of their occurrence in syphilis that Baumgarten stands practically alone with his view. Beitzke quotes two of his three cases which show that giant-cells are rare in old gummas, but are numerous in fresh gummas.

In text-books, the word "gumma," or "gummatous," is invariably linked with the term syphilitic meningitis and the impression is that syphilis of the meninges means gummas in the membranes. On the other hand, in journals there are reports of syphilis of the meninges with no mention of gummas. This discrepancy proves however to be only apparent, for the term "gumma," or "gummatous," is used rather vaguely. What one writer describes as a diffuse infiltration, another refers to as a gummatous infiltration, and a third one calls gumma.

Ziegler¹¹ speaks only of gummas and syphilitic foci: "At the beginning of the disease a circumscribed inflammation occurs in the pia and the subarachnoid tissue which soon leads to the formation of a gray, or reddish gray, slightly translucent, sometimes gelatinous, granulation focus. The gummatous foci may be simple or multiple. A single focus may be very small—more frequently, however, there are larger foci, called gummas. On the surface of the cerebrum they generally extend in the sulci and consequently show their configuration. In the fossa Sylvii they occur in the form of streaks, while over the brain and spinal cord they form variously shaped, flat foci. In rare cases, a more diffuse infiltration of the membranes at the base of the brain has been observed." Adami¹² mentions the formation of gummas and the involvement of the vessels as the two main features of syphilitic leptomeningitis. "The gummas may be single or multiple and are not infrequently localized in a comparatively small district. It is moreover not uncommon to find definite large masses of them or diffuse gummatous infiltration." Kaufmann¹³ describes basilar gummatous meningitis as the usual form of brain syphilis, consisting in the formation of circumscribed solitary or multiple specific inflammatory foci or in a more diffuse specific tissue formation within the membranes. Nonne⁴ states: "Where the meninges become involved primarily we have the gummatous form, and this gummatous change reveals itself either as a diffuse infiltration or as a miliary focus within the fibrous thickenings of the membranes." Naturally, there are authors who question the propriety of using the term gumma for conditions fundamentally the same but which differ considerably in microscopical appearance. Meyer¹⁴ writes: "The gummatous infiltration of authors is no gumma in the proper sense. According to the view of many, it is true, the diffuse cellular new formation forms the anatomical basis of the gumma, but diagnostically

9. Hygiea, 1894, 56, p. 85.

10. Deutsch. Ztschr. f. Nervenhe., 1910, 39, p. 459.

11. Lehrbuch der speziellen pathologischen Anatomie, 1892, p. 369.

12. Principles of Pathology, 1911, 2, p. 575.

13. Lehrbuch der speziellen pathologischen Anatomie, 1911, 2, p. 1162.

14. Zentralbl. f. allg. Path. u. Path. Anat., 1898, 9, p. 746.

there is undoubtedly a considerable difference between the more or less sharply delimited new formation with the various metamorphological forms and, for example, the diffuse cell infiltration which extends throughout the entire pia Where the tendency to circumscribed tumor formation within the diffuse infiltration is very pronounced, the term gummatous infiltration might be retained."

Another point of discussion is the question of the primary natures of gumma and gummatous infiltration and meningitis and gumma in the brain. Boettger¹⁵ believes that the gumma does not result secondarily from the cellular infiltration but that the latter frequently follows the gumma. Quincke¹⁶ states: "The longer lues exists the more does the meningitis become circumscribed and, at the same time, poorer in cells; the terminal phase is the meningeal gumma; between this and the initial serous meningitis there are many intervening stages . . . but the meningeal gumma like any other focal lesion, may temporarily give rise to an acute serous inflammation." Virchow, Heubner, Buttersack, and Schultze consider a "simple meningitis" the result of regressive changes of gummatous processes.

The meningeal origin of gummas in the brain is practically agreed upon by all, and also that the gummas may again be the origin of a meningitis secondarily. There is no connection between the gummas of the brain and the specific nerve tissue, tho the latter is nearly always affected secondarily more or less severely.

The second type of change, chronic hyperplastic inflammation is probably present in some degree in all cases of syphilitic meningitis. The presence of fibrous thickening in the form of more or less extensive plaques, gray, white, or yellowish, located in various regions of the brain, or microscopically an increase in the connective tissue is generally mentioned but not discussed in descriptions of syphilitic meningitis.

Some authors claim that the changes in the meninges due to alcoholism may appear similar to those from chronic syphilis. They assert that the plaques of thickening found on the convexity, chiefly along the intercerebral fissure, and the grayish, whitish, or yellowish white masses of opacity in the pits formed by sulci are found as well on the brains of chronic alcoholics as on those of syphilitic subjects. This assertion, to be of any value, must be founded on actual observations supported by very accurate, clinical data and reliable histories. Of course, alcohol is one of the most important contributing causes of death in cerebral syphilis, for example, Tarnowsky¹⁷ found that of one hundred individuals with syphilis of the central nervous system, twenty-nine were cases of neurasthenia; six of overwork; five, head traumas; and forty-three alcoholism. Many writers (Oppenheim, Kahane, and Hjelmman) state that alcoholic excess or chronic alcoholism is the history in many cases of brain syphilis. It is difficult to see how in these cases a separation of lesions from both sources could be done with any accuracy. As both affections are of a decidedly chronic nature, one resulting from the constant reintroduction of alcohol into the system and the other due to the constant flaring up of the infectious foci in the system, it must be supposed that the lesions are intermingled and superimposed. The physiognomy of alcoholic meningitis cannot be learned from hybrid forms, and therefore the lesions of purely alcoholic and purely syphilitic origin must be sharply separated from those of chronic alcohol-

15. Quoted from Meyer: *Zentralbl. f. allg. Path. u. path. Anat.*, 1898, 9, p. 746.

16. *Deutsch. Ztschr. f. Nervenh.*, 1909, 36, p. 379.

17. *Arch. f. Dermat. u. Syph.*, 1891, 23, p. 384.

ism in syphilitic individuals, on the one hand, and of brain syphilis in alcoholic subjects, on the other.

The histological picture of alcoholic meningitis which Meyer¹⁸ gives would rather indicate a decided morphological difference between the two conditions: "The thickening of the pia is a hyperplastic, and not an infiltrative, condition. There is an increase in the connective tissue which occurs in masses of loose fibers; the scattered cells appear morphologically as connective tissue cells. Plasma-cells and lymphocytes, in considerable number at any rate, cannot be demonstrated." Other accounts mention vascular changes, as fibrous thickening of the wall and hyaline degeneration in the pia and brain substance and a slight periarteritic increase in the cells. In a monograph by Rose,¹⁹ in 1884, on delirium tremens there is no account of any special meningeal changes due to alcohol, and his observation was that the characteristic feature of acute alcoholism (delirium tremens) is edema, not turbidity of the meninges. He states: "Where death occurs in delirium tremens itself, edema is the marked feature; the more often delirium tremens repeats itself, the longer all the forms of chronic alcoholism persist, the greater is the turbidity of the pia and the atrophy of the brain."

Animal experiments with alcohol poisoning do not convince us that there is any particular similarity in the lesions produced by the alcoholic poison and those caused by the syphilitic virus. First of all, it must be mentioned that the results of these experiments have not been uniform. The microscopic changes which Lissauer²⁰ found in two of four rabbits were perivascular infiltrations, consisting of lymphocytes and plasma-cells. There were no gross lesions in the meninges of these two animals. In the other two rabbits, the membranes were normal. Montesano²¹ reported a perivascular infiltration with plasma-cells. Ferrari²² found thickening and infiltration of the meninges in guinea-pigs fed and injected with alcohol and similar changes in the offspring of the animals. Lissauer mentions the changes found by other investigators, as by Braun of an inflammatory infiltration of the pia, by De Rechter of hyperemia and thickening, by Afanassajew of fat infiltration and cells with fat granules in the pia, and by Ruge of engorgement of the vessels and slight edema. Fahr²³ found no changes macroscopically or microscopically in eight guinea-pigs which had been fed with alcohol for two years.

Whether or not the involvement of the vessels is to be considered the direct starting point of syphilitic processes in the meninges and secondary to those in the brain, changes within or around the vessel walls are as a rule a conspicuous feature of syphilitic meningitis. Without entering into the controversies regarding the specificity or non-specificity of the vascular lesions found in syphilis of the nervous system, it may be stated that Heubner's endarteritis has not been observed unaccompanied by chronic meningitis except with tumor. This disease is frequently syphilitic on account of certain peculiarities, such as the absence of calcification, caseation and fatty degeneration, the tendency to focalization, and the splitting or reduplication of the elastica interna. There is some difference of opinion as to whether the doubling of the elastica is due to the splitting of the old one or to the formation of a new one and where the starting point of this process is to be sought. Marchand believes that the second elastica

18. Quoted from Lissauer: *Centralbl. f. allg. Path. u. path. Anat.*, 1913, 24, p. 337.

19. *Delirium Tremens und Delirium Traumaticum*, Deutsch. Chirurgie, Stuttgart, 1884.

20. *Centralbl. f. allg. Path. u. path. Anat.*, 1913, 24, p. 337.

21. *Centralbl. f. Nerven- u. Psychiat.*, 1907, 18, p. 849.

22. *Monatschr. f. Psychiat. u. Neurol.*, 1910, 28, p. 483.

23. *Verhandl. d. deutsch. path. Gesellsch.*, 1909, 13, p. 162.

is formed by a splitting of the old. Dürck convinced himself, by Weigert's stain for medullary sheaths, that the new elastica is not a splitting process but a new formation, independent from the old elastica. This author also expresses the view, that it is not the endothelium itself which participates in the proliferation of the intima, as Heubner believed, but the so-called cellular layer, which is situated between the membrana fenestrata and the endothelium. According to him, the giant-cells, which are nearly always in the immediate neighborhood of the elastica interna, start from the Langhans' layer.

With all the similarity between tuberculous and syphilitic lesions in general, there are nevertheless rather distinctive features in the vascular changes in these two affections. A clear distinction of the cerebral vessel changes in syphilis from those in tuberculosis is made by Beitzke,²⁴ who states: "In syphilis . . . we have in the main a panarteritis, a cell infiltration consisting chiefly of round cells and starting from the adventitia or the adventitial lymph sheath; and anatomically independent from it a proliferation of the intima, consisting chiefly of cells with oval and spindle shaped nuclei; occasionally giant cells are found. Sometimes, however, the cell infiltration of the external coats breaks through the elastica interna, when it spreads in a fungus-like manner along the inner surface of the elastica; but the round cells of the granulation tissue can well be distinguished from the larger oblong cells of the thickened intima. If the process is advanced, only this thickening of the intima remains, with complete obliteration of the lumen, as the case may be. The infiltration of the media and adventitia has disappeared, leaving only small scars."

"In tuberculous meningitis there are likewise cell-infiltrations in the adventitia and media, which, however, consist chiefly of leukocytes with lobulated nuclei, even in chronic cases. Proliferation of the intima of the pial vessels is seen only to a limited extent; probably an obliterative tuberculous endarteritis scarcely ever occurs in the cerebral arteries. The occlusion is here produced by the tuberculous granulation tissue penetrating from without, which at first only lifts the endothelial membrane, then fills out the entire lumen, and finally undergoes caseation together with the arterial wall. The elastic fibers behave in an entirely different manner in the two infections. In chronic tuberculosis, the elastic membranes are generally totally destroyed or to a few remnants in the caseated portions, while in syphilitic caseation they can as a rule still be very nicely demonstrated. The formation of a new elastica interna, so frequent in syphilis, is never observed in tuberculous arteritis cerebialis."

Lesions in the arteries are not a constant feature nor are they the only vascular changes in the syphilitic processes of the meninges. Little is known regarding changes in the veins. In Stursberg's⁸ case the arteries of the pia were only slightly involved in the inflammatory process, there were a few round cells in the external layers of the adventitia and no changes in the media or intima. The veins, however, were thickened, and infiltrated with round cells. Strüssler²⁵ found a marked infiltration both in arteries and veins in a case of syphilitic meningitis of the convexity in juvenile dementia paralytica.

A very interesting report from this standpoint was recently made by Versé²⁶ concerning a marked involvement of the veins of the convex surface of the under surface of the right temporal lobe and at the border of the left cerebellar hemisphere; microscopically, there was also a marked infiltration of the veins of the spinal cord. The case is particularly interesting as numerous spirochetes

24. Jour. Exper. Med., 1896, 1, p. 467.

25. Ztschr. f. d. ges. Neurol. u. Psych., Orig., 1912, 12, p. 365.

26. Beitr. z. path. Anat. u. allg. Path., 1913, 56, p. 580.

were found in various places in the walls of the veins, which furnished the author conclusive evidence of the mode of the invasion of the organisms. The rather frequent involvement of the cerebral veins may have a significance analogous to that given by some writers to the syphilitic involvement of the veins in other parts of the body. Rieder²⁷ from his study of seventeen cases of syphilitic stricture of the rectum, all marked by an extensive involvement of the veins with an almost complete intactness of the arteries, made this statement: "The syphilitic virus evidently has the property, not only of producing pathological processes in the places where it settles, but it attacks at once the vessels (veins and lymph channels) and progresses in nearly every case. The venous sclerosis marks, so to speak, for some distance the path on which it travels from the place of its entrance on to new foci and to dissolution in the general blood stream." A remark of Nonne is to this effect: "Cases suggest the thought that also in the central nervous system the veins are first involved specifically." Versé's report seems to be a confirmation of Nonné's supposition.

As regards the lymph channels and the part they play in syphilitic affections of the meninges, Koester believes that the great thickening of the intima of the arteries may be due in part to an impediment to the conveyance of the nutrient material, since the lymph spaces are often displaced or also entirely blocked by inflammatory proliferations. Others, including Baumgarten, Friedländer and Ranvier, share this view. It is generally believed that the starting point of syphilitic processes is in the intra-adventitial and extra-adventitial lymph-spaces. The reciprocal effects from disturbances in the lymphatic system and processes that impede the blood circulation are probably of very far reaching consequences. Binswanger²⁸ has shown how the vessel changes and the resulting disturbances in the intra-adventitial and extra-adventitial lymph-spaces in paralytic dementia are intimately connected with the lesions in the nervous system. He demonstrated that the meningitis, which is present in most cases, and the hydrocephalus internus may also be attributed in a large measure to the alterations in the lymph stream, since the epicerebral space communicates with the intravascular and extravascular lymph-spaces and the former becomes obliterated where the pia becomes adherent to the cortex. Finally, it is possible that the chemically altered waste-products, which are eliminated from the vessels into the lymph-sheaths, injure, anatomically and functionally, the walls of the lymph channels and the nerve elements. Nonne⁴ expresses practically the same view. Dürck⁵ makes the frank statement: "The origin and spread of syphilitic meningitis is exclusively by way of the lymph channels and is not, as in tuberculosis, only a part of the phenomena of a general haematogenous nodular eruption."

The changes which have been described as characteristic of syphilis of the meninges, i. e., the gumma and the vascular changes, are processes which are generally classed among the so-called tertiary lesions of syphilis. Nowhere are the shortcomings and incongruities of Ricord's division of the lesions of syphilis more evident than in syphilis of the central nervous system. Neither have the attempts at classification by others been successful. As long as it was believed that syphilitic affections of the nervous system and its membranes were late manifestations of a syphilitic infection and that the rare exceptions were to be regarded as precocious cases, the fallacies of Ricord's, as well as Hutchinson's, division were not so obvious. But since the observations of a number of

27. Arch. f. klin. Chir., 1897, 55, p. 730.

28. Virchows Arch. f. path. Anat., 1898, 154, p. 389.

investigators have demonstrated that the contrary is more often the case, i. e., that syphilitic involvement of the central nervous system occurs very frequently at an early period of the infection, the confusion becomes almost distressing.

As Ogilvie²⁹ expresses it, "We are led to the paradox that a large majority of the tertiary phenomena occur in the secondary stage." Therefore, it seems best to avoid the terms "secondary" and "tertiary" in connection with brain syphilis, both in regard to time and to the pathological characteristics, for to quote Ogilvie again: "Another point of importance elicited from the post mortem statistics is that, with the exception of tabes and general paralysis, no syphilitic disease of the nervous system and membranes is peculiar to a certain age of the infection. During the first year after infection, gummatous disease and disease of the blood vessels with its consequences are not rare. On the other hand, disease of the baso-cranial nerves, generally considered of comparatively early appearance, is often met with in the latest stage. This result entirely coincides with the experience of Dr. Gowers, who says that disease of the arteries might occur at any time between the first and the twenty-fifth year after the chancre, altho usually before the seventh or eighth year, that whenever it occurs it is exactly the same in appearance, and that the gummatous lesions are less common, but are found at the same period of syphilis as the arterial lesions."

We have mentioned that very recently a classification of syphilitic meningitis upon the basis of the general classification of syphilis was attempted by Sézary¹ who believes that the same pathological characteristics and pathological tendencies which form the foundation of the latter can also be observed in the forms of syphilis of the meninges. "Secondary" syphilitic meningitis is subdivided by him into "latent," "frank" (avérée), and transitional, or "abortive" (fruste), forms. The first is only revealed by lumbar puncture and appears at the moment of the general eruption. According to Ravaut³⁰ its intensity is parallel to that of the eruption and it occurs in at least half of all cases of syphilis. The second form, "frank" meningitis, is rarer and corresponds to the acute syphilitic meningitis of other authors. The histological lesions are similar to those of the latent form. The general character of the lesions of tertiary meningitis corresponds to that of tertiary lesions; the author calls this type "scléro-gommeux." According to Sézary there is a similar relation between secondary and tertiary syphilitic meningitis as between the macules and papules and the gummas. A third type is called "residual," or "meta" syphilitic, or "parasyphilitic" meningitis, and is characterized by residual lymphocytosis which, having gradually diminished, arrives at a certain rate below which it cannot be brought, even with intense treatment. The meningitis associated with such a "residual lymphocytosis" is not the same as tertiary meningitis; it is not syphilitic according to Sézary, chiefly on the ground that treatment does not cure it, tho it is possible for it spontaneously to disappear slowly, like any other chronic meningitis, in the absence of treatment. Very probably, it is purely irritative and due to an inflammation kept up by the neighboring cicatricial focus and favored by the former meningeal lesions.

This classification would seem to lend meaning and precision to terms which apparently are so often ill-applied to syphilitic lesions of the central nervous system. The views expressed by the author leave room for discussion, however. If "secondary meningitis" sets in at the moment of the general eruption and the condition of the membranes during the secondary period depicts the general

septicemia, or spirochetemia, the question arises why serous membranes other than the pia-arachnoid, for example, the pleura and the pericardium, are not similarly affected by the blood infection? Sézary states that "frank" syphilitic meningitis, characterized like the other forms of secondary syphilitic meningitis by generalized diffuse lesions with a tendency to resorption, corresponds to the acute syphilitic meningitis described by many authors. In consulting the literature on this point, we find that some of the reported early, acute cases have this diffuse infiltration, but other, and perhaps more, cases have circumscribed lesions in addition to the diffuse infiltration. A number of the group "nodular syphilitic meningitis" are acute cases. It is very questionable whether any sharp lines can be drawn. Sézary distinguishes the lesions of secondary syphilitic meningitis from those of tertiary syphilitic meningitis by the tendency of the former to resorption and of the latter to sclerosis and necrosis. If we admit that in many cases of secondary syphilitic meningitis resorption takes place, we must explain why all cases having the same tendency do not terminate in this manner. We have no proof that "tertiary" syphilitic meningitis starts entirely independent from any involvement of the membranes during the secondary period of syphilis and that its beginning, course, and termination are entirely different from those of "secondary meningitis."

Objections might also be raised against Sézary's views concerning "residual," "metasyphilitic" or "parasyphilitic" which, for reasons mentioned, he does not consider syphilitic. In the first instance, the terms "metasyphilitic" and "parasyphilitic" are, in such cases, out of place. Apart from the fact that these terms have lost their meaning and are being abandoned since the general recognition of the truly syphilitic nature of such conditions as tabes and dementia paralytica, the accompanying meningitis is purely irritative, and is neither syphilitic nor parasyphilitic. The new term, "residual," introduced by the author, which expresses its chief feature, "residual lymphocytosis," does not seem more appropriate, as it fails to emphasize the non-syphilitic nature of this type of meningitis. "Residual" still connects it with syphilis, of which it retains a residue, and "metasyphilitic" and "parasyphilitic" link it to metasyphilitic diseases or diseases now known as syphilitic.

We refrain from discussing the meningitis so frequently associated with the so-called "parasyphilitic" diseases. We shall merely mention some reports³⁰ of dementia paralytica in which true syphilitic lesions of the central nervous system and true syphilitic meningitis were found simultaneously with the lesions of paralysis.

The frequent and early involvement of the meninges in the course of syphilis has been recognized only in recent years and chiefly through the examination of the cerebrospinal fluid. Since the use of the Wassermann reaction we have become impressed by the great number of individuals with syphilis of the nervous system. Desueux, Dujardin and Weill³¹ recently reported one hundred and sixteen cases of syphilis of the meninges observed within a period of twelve months. In some, the meningeal involvement was revealed by the "neurorécidif" reaction from the lumbar puncture and the reaction from treatment with salvarsan. The authors emphasize in particular the early involvement of the meninges in the course of syphilis. From the time of the generalization, i. e., about the

30. Strüssler: *Monatsschr. f. Psychiat. u. Neurol.*, 1906, 19, p. 244; *Ibid.*, 1910, 27, p. 20; *Ztschr. f. d. ges. Neurol. u. Psych.*, 1912, 12, p. 365.

Plaut: *Allgem. Ztschr. f. Psychiat.*, 1909, 66, p. 340.

Landsbergen: *Monatsschr. f. Psychiat. u. Neurol.*, 1911, 29, p. 147.

Giljarowsky: *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1911, 6, p. 21.

31. *Jour. de méd. de Bordeaux*, 1913, 18, p. 175.

third week, the meninges are affected with a peculiar constancy, and syphilis at this time is a real septicemia which attacks chiefly the skin and the nervous system. Gennerich,³² from examination of the cerebrospinal fluid during primary syphilis, stated that the meningeal infection is evidently of general occurrence in fresh syphilis since the fluids from these cases, examined during the course of treatment, revealed a slight modification.

The realization of this relation of the involvement of the central nervous system to syphilis came gradually. In 1786, Hunter³³ stated that he had never seen a case in which the brain was affected by syphilis and Astley Cooper³⁴ believed that the brain was of those tissues which are not influenced by the venereal poison. About one hundred years later, Julien furnished statistics on tertiary syphilis and had 22 cases with nervous involvement in 224 cases of tertiary syphilis. Fournier, in 1890, published an account of 1,085 cases out of 2,600, in which syphilitic lesions were referable to the central nervous system. In 1892, Hjelmman gave statistics from 1,860 cases of tertiary syphilis, collected from all the divisions of the general hospital in Helsingfors, 1878-1890, in 254 cases of which the central nervous system was affected, and in 218 the brain alone. Tabes and general paralysis were not included in the list. Haslund reported 514 cases of tertiary syphilis, 133 cases among which involved the nervous system.

Hjelmman discussed the question of the increased frequency of brain syphilis and stated that syphilis itself has not increased, altho descriptions from older writers suggest the contrary. An actual increase in the frequency of syphilitic diseases of the nervous system, however, is believed by a number of writers. The cause of this increase is thought to be due rather to a change of the nervous system than to an increase of syphilis. The greater demand that is made on the brain for gaining a livelihood is thought to have influenced the nervous system, so that from generation to generation it yields more readily to the effect of the syphilitic infection. The relations between the inflictor and the inflicted seem to have changed in several respects. Bone lesions occupied at one time the first rank among the "tertiary" lesions. Sternberg, in 1860, writes: "Affections of the bones are becoming relatively rarer; on the other hand, syphilis seems to be more ruinous, graver, since it seems to attack now the inner organs, especially the brain, much more frequently." Also, Rumpf and others have called attention to the fewer bone lesions. The view expressed by older writers that syphilis of the central nervous system starts from lesions of the bones of the skull is in all likelihood based on erroneous premises and has nothing to do with the former observation. By later writers, no such relationship has been found by post mortem examinations.

Statistics of the frequency of brain syphilis cannot of course be made, but the statistics of the relative frequency are of more value. Fournier gives the following: tertiary syphilides, 787 cases, subcutaneous gummas, 428; tertiary lesions of the genital organs, 157; tertiary lesions of the tongue, 152; tertiary lesions of the velum palati, 179; tertiary lesions of the pharynx, 71; tertiary lesions of various mucous membranes, 30; bone lesions, 336; lesions of the nasal bones and of the bony palate, 137; arthropathies, 14; gummas in tendons, 3; gummas in muscles, 12; lesions of the digestive canal, 4; anorectal syphilomas, stricture of the rectum, 5; lesions of the larynx and the trachea, 23; lesions of the lungs, 14; lesions of the heart, 2; lesions of the aorta, 6; lesions

32. Quoted from Jacob: *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1913, 19, p. 188.

33. Cited from Hjelmman's monograph: *On hjärnsyfilis, dess frekvens, kronologi, etiologi och prognos*. Helsingfors, 1892.

of the liver, 9; lesions of the kidney, 9; lesions of the testicle, 145; lesions of the eye, 69; lesions of the auditory apparatus, 8; cerebral syphilis, 461; "accidents cérébro-spinaux," 11; monoplegias, 3; spinal syphilis, 77; tabes, 355; cerebrospinal tabes, 45; muscular atrophies, 19; general paralysis, 32; insanity, 9; ocular paralysis, 57; facial hemiplegia, 13; various nervous affections, 3; various affections, 8.

Haslund examined 514 cases from the fourth division of the "Kommune" Hospital in Copenhagen. Here, also, "tertiary" syphilis is referred to: skin, 290 cases; nervous system, 133; bones, 131; mucous membranes, 96; inner organs, 22. Hjelmman's statistics from 1,860 cases are: lesions of the skin, 985 cases; lesions of the bones, 238; lesions of the nose and hard palate, 223; lesions of the pharynx and soft palate, 318; lesions of the larynx and trachea, 93; lesions of the genital organs, 50; lesions of the testicles, 32; lesions of various mucous membranes, 15; lesions of the eye, 32; lesions of the joints, 10; lesions of the liver, 32; syphilis of the brain, 218; syphilis of the brain and spine, 12; syphilis of the spine, 24; various lesions, 2.

In eighty-eight cases, Petersen found lesions in the liver in seventy-seven cases, in the kidneys in thirty-four, and in the brain in nine. Bernheim and Eichhorst state that of the inner organs only the liver competes with the brain. Nonne⁴ gives the order in the frequency of the lesions as: arteries, kidneys, liver, and brain. According to Mauriac and Breus the greater number, according to Gaudichier and Hjelmman more than one-fourth, of the cases develop in the first year of the infection. Naunyn,³⁴ from the examination of three hundred and twenty-eight cases, came to the conclusions that 48 percent of the diseases of the central nervous system occur during the first three years, that after the number decreases from year to year, until after ten years the nervous system is only exceptionally involved. Rumpf³⁵ found that of forty cases of syphilis of the central nervous system nine developed within the first year. Mingazzini³⁶ reported eighteen cases from the literature and five of his own cases, in all of which cerebral manifestations occurred between one and one-half months to one and one-half years after the infection. Brasch³⁶ observed a case of brain syphilis which occurred six weeks after the primary lesion, while Nonne reported severe cerebral phenomena appearing with the secondaries. Quincke³⁷ reported a death from myelitis and cerebrospinal meningitis with clinically and anatomically a four-day course. The luetic infection had occurred three months previous to this. There was a slight, fresh leptomeningitis with hemorrhages into the meninges of the anterior part of the brain. Stursberg³⁸ observed severe nervous manifestations four months after the primary lesion with death two months later. There were still distinct signs of roseola. The microscope showed an extensive leptomeningitis with numerous small foci of softening in both hemispheres, caused by thrombosis of the smaller arteries. Two interesting cases are reported by Löhe.³⁸ In the first, the nervous involvement manifested itself three months after the initial lesion, when secondary skin lesions were still present, and, in spite of antisyphilitic treatment, death occurred. In the second, cerebral symptoms, as hemiplegia, occurred as early as twenty-four days after the primary infection before there were even slight secondary manifestations, while there were still the initial sclerosis and multiple indolent glandular swellings.

34. Mitt. a. d. med. Klinik z. Königsberg i./Pr., Leipzig, 1888; Berl. klin. Wchnschr., 1888, 25, p. 870.

35. Quoted from Löhe: Berl. klin. Wchnschr., 1910, 24, p. 1127.

36. Deutsch. Ztschr. f. Nervenhe., 1896, 8, p. 418.

37. Ibid., 1909, 36, p. 343.

38. Berl. klin. Wchnschr., 1910, 47, p. 1127.

Abnormalities in the cerebrospinal fluid, such as increase in the number of cells or in the protein content, have been found in primary syphilis. Altmann and Dreyfus³⁹ examined eight cases, in two of which there were positive Nonne-Apelt reactions, Phase 1, and cell-counts of eight and thirteen, respectively. In secondary syphilis the changes are frequent. Ravaut⁴⁰ examined one hundred and sixteen cases and found some abnormalities in 67 percent. Dreyfus⁴¹ reported changes in all of the twenty-two cases which he tested. Fränkel⁴² in fifteen cases of early untreated secondary syphilis, found positive Wassermann reactions in five, Phase 1 in one, lymphocytosis in five, and counts of four to ten cells in four cases. In fifty-six cases of untreated secondary syphilis Altmann and Dreyfus³⁹ reported some increase in cells and globulin in 66 percent. Seven of these cases had cell counts of between seventy-five and one hundred cells, showed marked increase in the globulin content, and gave positive Wassermann reactions. This reaction was positive in five other cases of this group. Ellis and Swift⁴³ found abnormalities in 36 percent of twenty-two cases of untreated secondary syphilis and 100 percent in secondary syphilitic meningitis; four of these eight cases were treated.

Concerning the relation of dementia paralytica to the meningitis which so frequently accompanies it, a number of authors consider the anatomical lesions in this disease the result of a syphilitic condition of the lymph channels, blood-vessels, and meninges. The demonstration of spirochetes in the meninges as an aid in the diagnosis of the syphilitic nature of meningitis has so far played only a small part, as search for them has rarely been successful. In hereditary syphilis they have been found repeatedly. Strassmann, in 1910, was the first to demonstrate the organisms in the brain and spinal cord in acquired syphilis; Beitzke found them in one of three cases in 1911. In Strassmann's case they were seen wherever the inflammatory changes were dominant, in the large arteries, around the vasa vasorum, in large numbers in the adventitia and muscularis, very sparingly in the proliferated, not infiltrated, intima. They were always found in the proliferated vessel-walls of the diseased portions of the brain and medulla and were seen free in the tissue of the diffusely infiltrated and thickened meninges and in the infiltrated septa extending into the brain and spinal cord. "Their chief path of spreading," Strassmann states, "is at any rate connected with the course of the small vessels, in the lymph-sheaths and proliferated walls of which they apparently multiply." The spirochetes were found exclusively in the central nervous system. In Versé's case, reported in 1913, a few were seen in the testicles, but none in the other organs. Strassmann lays particular stress on the importance of the lymph stream for the multiplication and spread of the organisms. A similar view was expressed by Ranke who found spirochetes in the central nervous system in a number of syphilitic newborn children. Noguchi⁴⁴ who recently was so successful in finding the organisms in a large percentage of the brains of dementia paralytica, due to his modification of the Levaditi method, states that they were very rarely in the neighborhood of vessels or the vessel-walls, and that they were searched for in the pia, but could not be demonstrated with certainty.

Vauzetti⁴⁵ reported the transplantation of syphilitic material from the testicle of a rabbit into the subdural space of the brain of a rabbit, which resulted

39. München. med. Wehnsch., 1913, 60, p. 264.

40. Rev. mens. d. méd. int. et de thérapeutique, 1909, 1, 275.

41. Quoted from Ellis and Swift: Jour. of Exp. Med., 1913, 18, p. 162.

42. Jour. Exper. Med., 1913, 18, p. 162.

43. München. med. Wehnschr., 1913, p. 737; Jour. Exper. Med., 1913, 17, p. 232.

44. Arch. p. le sc. med., 1914, 38, p. 1.

in lesions in the dura, leptomeninges, and the brain substance near the inoculated fragments and at some distance and even in the other hemisphere, which were similar to lesions considered syphilitic in human brains. Search for spirochetes was always successful with reference to the transplanted pieces and always negative with regard to the lesions in the meninges and the brain. Noguchi⁴⁵ has also reported experimental work bearing on this question.

The following report is the result of the study of gross alterations in the meninges, or of changes visible with a low-power hand lens. As the changes in the meninges were frequently associated with syphilitic lesions elsewhere in the body, we believed that the meningeal lesions might have a syphilitic origin. The brains were not selected because there were luetic lesions elsewhere in the body, but all the brains possessing such changes were examined. These brains were found in successive post mortem examinations which extended over two years and five months. Mention is made of only the most significant changes in the anatomic diagnoses, and the lack of clinical details is due to the fact that, in most cases, the examinations (E. R. L.) were made for the coroner of Cook County on bodies of persons dying unexpectedly and without medical attendance. With only a few did the conditions permit of careful clinical observation and diagnosis. Unless otherwise stated, the interiors of the brains were found to be free from changes.

There are three main types of changes in the pia: opacity or turbidity; patches of fibrous thickening; and discrete focal lesions. Turbidity of the pia in some degree and extent is present in nearly all cases. The turbidity may be general and extend over almost the entire brain, shrouding it as with a veil or a sheet; the occipital lobes are usually unaffected. Sometimes, the turbidity appears only in patches, which are separated by portions of normal pia, while occasionally it is limited to one or two areas, and these may be small. Often the base is entirely free, but when it does exist there, it is marked over the pons, the interpeduncular space, and the region of the oculomotorius. The degree of opacity is also variable; it may be very slight and again it may be so marked that all the markings of the surface are masked, the sulci bridged over, the vessels only recognizable as dim, vague lines of a darker hue. When the opacity is prominent, the brain, if fresh, has a bluish white, dull hue, and appears as tho milk had been poured over it (Fig. 1). Otherwise it is grayish or pinkish gray. According to our observation, the opacity is much more frequently found over the con-

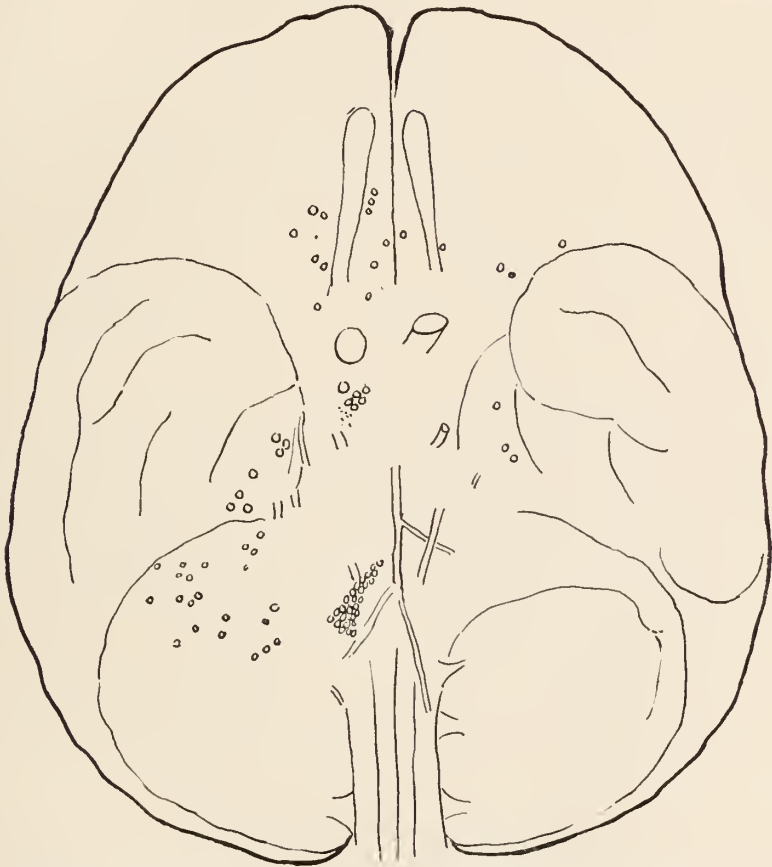
45. *Presse Méd.*, 1913, 8, p. 805.

vexity than on the base. It is generally pronounced over the fossa Sylvii; indeed, the two fossae or only one may be the chief or only locality where the pia is opaque.

Very frequently the second type of alteration, patches of fibrous thickening, are found in addition to the turbidity of the pia. These may appear as areas of a more or less diffuse white, or grayish white, dense thickening, more often along the intercerebral fissure, where it may extend along the entire length, except the occipital lobes as a rule, often being densest in the area bordering the median line. Frequently, there are circumscribed plaques of tough, dull white, or a glossy, yellow, bacon-like thickening, 1, 2, and 3 mm. in depth and 1, 2, and more cm. in length or width, also preferably in the area alongside the median line, the favorite seat of the pacchionian bodies, which may be found above, beside, or partially covered by them. Patches of a grayish, gelatinous, somewhat succulent thickening, generally of smaller dimensions, the size of a pea, bean, dime, or larger, may be seen in any locality, more on the convexity, not infrequently on the anterior pole of the frontal lobes, at the base on the anterior portion of the temporal lobes, and on the orbital lobes. These gelatinous patches are often located over gaps between convolutions and, when small, partake of the configuration.

The recesses between convolutions, where an opaque and more or less thickened pia stretches across and levels them with the summits of the gyri, are also the places of predilection of a third type of lesions. These are circumscribed, homogeneous, grayish white, white and yellow, slightly greenish, or yellow masses of thickening of a denser consistency, either deeply or superficially imbedded within the pial thickening, and distinct or very dim according to the thickness of the film covering the lesions. They may be vaguely outlined and assume, in a measure, the form of the pit and be round, triangular, or stellate, 2, 3, and 4 mm. in diameter, or they may be well defined, round, nodular, flat or elevated, miliary and submiliary, very distinct or only clearly seen with the hand lens, more frequently single, but also in groups of two and three. They are found in any locality of the convexity. If the opacity of the pia is not too dense these nodular masses are often seen over a vessel. A homogeneous, dense, white or yellowish white thickening is also frequently found in the form of streaks and bands accompanying the vessels. The favorite locality of these forms is the fossa Sylvii which is often leveled by an opaque thicken-

ing; occasionally, larger, circumscribed masses of thickening, identical in color, consistency, and general appearance to the former, are seen as superficial, elevated areas, sometimes with raised crenated borders, irregular in shape and following the course of a fissure. Nodular elevations are arranged bead-like in short chains or in ridges.



Text-Figure 1.—Diagrammatic representation of the base of Brain 48 which in addition to the basilar fibrinous exudate, the lesion generally described in accounts of syphilis of the meninges, showed miliary or slightly larger gummas distributed, as illustrated. Structures not shown were covered by the exudate.

Finally, in a few cases the raised, sessile, rarely pedunculated nodule occurs (Text-Figure 1). With strictly basilar meningitis they are all of the miliary type, very small, in groups, and closely apposed. Under the hand lens, however, they are easily discerned as discrete

nodules. They are in patches covering the root of the olfactory trunk, the optic chiasma, the oculomotorius, and over the interpeduncular space; scattered, singly or in small groups, over the temporal lobes and the cerebellar hemispheres. On one brain the nodules, which are exceedingly numerous and usually rather large, are present on the base in by far the largest number in the left fossa Sylvii, but also scattered in various places of the convexity. They vary in size from 2-4 mm. in diameter, are sometimes flat, with raised borders and as if stuck on, often with broad bases and conical, or clubbed, or other irregularly shaped summits. In the left fossa Sylvii they are so close together that they form dense aggregations, while upwards and downwards from the Sylvian fissure, stray nodules are found. The dura is adherent in this locality. On its inner surface there are several patches of closely apposed, or even fused, nodules.

Vascular changes at the base of the brain are not infrequent. The middle cerebral, the basilar, the posterior cerebral, and the vertebral arteries show rigidity and scattered yellow patches of thickening in their walls.

As incidental findings may be mentioned pial hemorrhages, which in one case tinged the entire convex surface a vivid red. In two or three cases, there were restricted areas of hemorrhage. Sometimes, a brain has a peculiarly mottled appearance from the simultaneous presence of patches of white fibrous thickening, areas with blood effusion, regions with a transparent clear pia, gray gelatinous patches, white lines bordering the dark engorged vessels, and small yellowish specks here and there shining through a gray, opaque pia.

Rarely, a brain has only one type of meningeal alterations; there may be only an opacity. Generally there are two and not infrequently all of the lesions are present on one brain. The opacity is generally combined with plaques.

On account of the special attention which the "nodular" ("Knötchenförmige") syphilitic meningitis has recently received, a word concerning the size and general appearance of this group of lesions may be added. The value of classifying this form of syphilitic meningitis as a special type rests upon its great resemblance, macroscopically, to tuberculosis and the necessity of differentiating it from the latter. Baumgarten³ took particular care in his case of syphilitic meningitis, with multiple gummas already mentioned, to separate the larger nodules from suspiciously small ones. The latter were scrupu-

lously searched for, but not found. The larger nodules, ranging in size from a hempseed to a cherrystone, were very numerous. In a subject who had both tuberculosis and syphilis, why were these lesions of the brain diagnosed as syphilitic when the small nodules in the lung were diagnosed without hesitation as tuberculous? Baumgarten's arguments for distinguishing the two are based on the size and appearance of the nodules. In his case, the nodules were nowhere smaller than a hempseed, and this was a point of importance to him.

Of this type in which there is a definite border line for the minimal size of the larger nodule, there is not a single example in our collection. Brain 32, which has numerous nodules with a maximum size of 3 mm., has also a great many smaller nodules, 1 mm. and smaller, both on the convex surface and on the base, some of which are discernible as nodules only with the hand lens. A single minute nodule is sometimes seen in the immediate neighborhood of a large nodule. All these nodules are raised.

Apart from the absence or presence of any microscopical difference, the situation of the nodules above or below the level of the surface constitutes a difference. When raised, they occupy chiefly the free surfaces of the convolutions on the base and other superficial structures, in one case, the olfactory nerve trunk. The non-elevated nodule is situated in the recesses formed by joining convolutions, and in this location it must be differentiated from another circumscribed lesion found there, a mass of thickening, not very well defined, imbedded in the opaque pia, and taking the configuration of the depression in which it lies, as triangular, stellate, or irregularly rounded masses, the processes not infrequently continued as white lines accompanying the vessels. The nodule, on the other hand, is always well defined, even when deeply placed and covered by a turbid film, and the hand lens will always show it as a distinct nodule, even tho two or more nodules are seen in one depression. Also, its form is independent of the shape of the recess. A direct connection between these nodules and the white lines following the vessels is not evident.

If we compare the gross changes with what has been generally described and adopt current terms, we come to these conclusions: Diffuse syphilitic meningitis, the common affection of the meninges, is very rare, and miliary syphilitic meningitis is not so rare as one is led to believe from the literature.

The histological changes we have found are on the whole in accordance with those in the literature. We should, however, remark that general statements, for example, that the diffuse gummatous meningitis at the base is the most frequent form of brain syphilis, can be verified no more by microscopic examination than by an examination of gross appearances. The tendencies of the granulation tissue in meningitis to diffuse spreading and to focalization cannot be strictly separated one from the other, any more than all the syphilitic brain lesions can be appropriately leveled to one group and marked "tertiary" lesions or appropriately divided into "secondary" and "tertiary" lesions. The stages from the extending infiltration to circumscribed round-cell accumulations and the formation of the gumma proper are "flowing" transitions.

Multiplicity and variability are the main characteristics of the pictures in microscopical preparations. All the stages and degrees of acute and chronic and of progressive and healing processes are encountered. The infiltration is slight, considerable, or marked; it is in the arachnoid around the large vessels of the subarachnoid space, in the pia, in the septa, and around the vessels of the cortex it is scattered, diffuse, or shows a tendency to focalization. One, some, or all of these conditions may be present on one brain.

There is a marked tendency of the infiltration to follow the perivascular lymph channels and the adventitial sheaths of the large vessels in the subarachnoid space, the perivascular spaces of the small vessels of the pia and in the cortex to have all degrees of round-cell infiltration. There is however no absolute regularity in this tendency, not even in the same brain. In the same sections it may be observed that some vessels are entirely spared, while those in the immediate neighborhood are surrounded with a dense coat of lymphocytes. Very good examples of such perivascular infiltration may often be found in sections through old necrotic gummatous areas (Figs. 20 and 21); these vessels stand out as concentrically arranged circular or elliptical areas, with the former lumen completely obliterated and now the site of a gumma, and the adventitia transformed into a greatly widened band of chiefly fibrous tissue intermingled with cells. In intermediate stages the cells predominate over the fibrous element in the adventitial layer. The vessels are intimately related to the infiltrating processes and the tendency to focalization is notable. The media is no selective place for infiltration, but scattered cells and small collections of cells are some-

times seen there. The intima also is not a favored site for lymphocytic infiltration; it may be entirely normal. When infiltrated, the round cells are generally scanty and often focalized when the same vessel may have a broad circle of lymphocytes in the adventitial sheath. Occasionally, the veins are the seat of inflammatory processes (Fig. 17), in fact, they may be involved heavily when the arteries are entirely, or almost entirely, spared. The cells in the proliferated portions of the intima of the arteries are of the type which Beitzke describes as different from those in tuberculous thickening of the intima; they are long and stellate cells with oval nuclei.

Giant-cells are not so rarely found; their nuclei are, as a rule, excentrically placed. They are generally in the close vicinity of the elastica interna and rather on the inner side; sometimes they are seen where the elastica is interrupted when they lie half way in the media, half way in the intima. Sometimes, they occupy an area which is bordered by elastic fibers on both sides. The lumina of the vessels are narrowed in various degrees. It is not at all rare to find one large proliferating area projecting from one pole leaving an excentrically placed lumen. A second elastica interna can easily be detected with the aid of special stains, but even the ordinary hematoxylin-eosin stain will sometimes show deeply curved zigzag lines which are not connective tissue. Cholesterol slits and cholesterol giant-cells may be found in the thickening of the intima.

Gummas occur in all sizes. We have studied them in sections cut through a mass projecting from the surface of the brain as a club-shaped nodule, more than 2 mm. in height, through an elevated nodule or ridge, the single nodule being not larger than 1 mm.; through minute nodules, slight elevations under the hand lens, through nodules on a level with the meningeal surface, and have found them in regions where no nodules were seen macroscopically. They represent all stages of formation and degeneration; gummas consisting of only a small, rounded mass of lymphocytes, without epithelioid cells and without any necrotic changes, others with slight, advanced or marked central necrosis with and without giant-cells.

Giant-cells differ in size, general appearance, and situation. Huge giant-cells are seen in a large necrotic gummatous area, not far from a region with a cellulofibrinous exudate; the spindle-shaped nuclei, closely apposed, occupy one-half or less of the peripheral circle. In sections from a fossa Sylvii, the seat of numerous large nodules projecting 2

mm. and more above the surface, the giant-cells present a most varied picture. Many of them are small, but their development through the fusion of epithelioid cells can be well observed. Some have only two or three darkly stained nuclei in one pole of a small area of homogeneous protoplasm, others faint outlines of individual cells and nuclei within the general protoplasmic cell-body. At one place, a rather large elliptical formation with a darker border zone and a more or less homogeneous center might at first sight be taken for a vessel, but in this gummatous area vessels are entirely lacking, while in a neighboring region within the cortex there are numerous vessels filled with blood. Moreover, with the high-power lens the border zone shows the nuclei distinctly. Part of the central area is homogeneous; some of it is occupied by faintly outlined cells or nuclei. In these sections the eosinophils are very numerous.

The fibrous thickening consists in the loosening and multiplication of the fibrous strands (Fig. 10), or in dense, compact masses of connective tissue. The maximum thickness is often opposite a fissure and diminishes as it reaches the summit of the convolution, but it is more generally in the upper portion, i. e., in the arachnoid. Lymphocytes and plasma-cells are found between the bands. The infiltration may however be considerable and rows of blue-stained cells may alternate with red bands of connective tissue (Figs. 11, 12, and 13).

The arachnoid is also frequently the seat of scars (Figs. 4, 6, 7, and 8). These are often lozenge shaped, are opposite the mouth of a sulcus, and vary in size and compactness. Some resemble keloids (Fig. 6) and are composed of rather broad, dense strands of connective tissue with a system of canals between them, recalling in this respect the canaliculization in the pachionian bodies, from which they are however entirely distinct. The process of healing which the scar represents, may be observed through all stages. In sections through the insula from a case of gummatous meningitis, scar formation is seen in the early stages: a central focus of an inflammatory process, varying in dimensions in the serial sections, is surrounded by broad bands of dense connective tissue. The whole process is limited to the arachnoid (Figs. 10, 11, and 12). In the meshes of the tissue beneath, there is a scattered infiltration which becomes somewhat denser towards the surface of the brain, which in these sections is at a great depth from the outer surface of the meninges. Microscopic preparations from the fossa Sylvii of the same hemisphere are chiefly occupied by gummas (Fig. 9).

Of cells, besides those of the connective-tissue type and their derivatives, the small lymphocytes always predominate. Plasma-cells are very frequently encountered, but always in restricted numbers, and they are always scattered. Eosinophils are present and are occasionally numerous, but polymorphnuclear leukocytes are rare and never numerous. Large and numerous phagocytic cells are met with occasionally, and in one instance of gummatous meningitis they were numerous in sections where there were no gummas. The huge cells, either in large aggregations or scattered, occupy the rather large spaces of the pia and arachnoid where also small groups of lymphocytes are found. The nuclei are large and generally multiple, two, three, and four. Small lymphocytes and other cell inclusions are found within them. Cells containing blood pigment are frequently seen. Fibrin is inconstant, and the amount is variable. It occurs as a serofibrinous or a cellulofibrinous exudate, or consists of a meshwork or single fibrinous shreds (Figs. 5 and 18), and we have found it apparently disappearing (Fig. 4).

In several instances, sections cut through what promised to be a focus of an inflammatory process or a fresh exudate, through dense, homogeneous, white or yellowish white, circumscribed masses in the recesses between convolutions imbedded in an opaque pia showed these to be nothing but thickening of the connective tissue strands of the pia arachnoid with but a slight infiltration, and what looked like a small well-defined nodule within a turbid or gelatinous pia in a sulcus or pit and which had been described as a nodule, or even definitely as a miliary gumma, proved to be only a scar with or without any infiltration. In one of these instances, the notion that there was an inflammatory process somewhere else in the brain was supported by finding a marked infiltration about some of the small vessels in the brain tissue. A piece from one of the fossae Sylvii was thereupon taken where there were no apparent focal lesions, simply an opacity of the pia, and histological examination showed a marked infiltration within the meshes of the pia. In another case, where again a "nodule" proved to be only ordinary fibrous thickening, the infiltration in the pia close to the brain and in the septa was of a character to arouse the suspicion that this case might be one of gummatous meningitis. The region of the fossa Sylvii was explored and microscopic gummas were found. After these experiences, when cutting pieces for microscopical preparations, one was always taken from the precentral convolution near or at the fossa

Sylvii whether or not there was any marked gross involvement of the pia here. Very frequently an infiltration was found.

In still another case in which all the data indicated gummatous meningitis, two discrete lesions of this type, taken from rather widely separated regions, were found to be scars. Gummas were found in sections from near the fossa Sylvii. In preparations from the insula, interesting stages of progressive infiltrative processes in the pia near the cortex with early stages of healing in the arachnoid were seen. The fossa Sylvii seems a lurking place for syphilitic processes, an easily accessible pathway for the infectious invasion.

As we consider the vast circonvolitional surface of the brain, all covered with pia which everywhere has the same rich supply of blood vessels and lymph channels so inducive to the extension of syphilis, we realize that the examination of a few places cannot in any way give a measure of the character or the extent of the involvement. In a few instances, these limited examinations are fair expressions of the general conditions, but more often additional examinations showed the brain in question to be a real mine of surprises. Even if the histological examinations in a large number of these cases were limited, they were possibly sufficient for the purpose of this study and have yielded valuable information in a general way. The fact that some of this information is best expressed negatively does not decrease their value, for example, a distinct nodule is neither always a gumma nor the seat of any active process; again, the external aspect of a brain cannot always be used as an indicator of the degree or type of syphilitic involvement; and gummatous or miliary gummatous meningitis of the convexity should not be diagnosed before the microscope justifies it.

Preparations were made from fourteen brains for the examination for spirochetes. The old Levaditi method and this method with Noguchi's modification were employed, and occasionally the Stern-Flexner method for smears was used. Typical gummas were found in six brains, but spirochetes in only Brains 32 and 48, tho not infrequently the arrangement of granular segments and other appearances were suggestive of the spiral curves of spirochetes. Many sections from lesions regarded as gummas were stained for tubercle bacilli, but none was found. In view of the generally recognized difficulty in demonstrating spirochetes in the central nervous system, even in the more acute lesions, the failure to find them in places where scars exist or are forming is scarcely surprising.

Therefore, absolute proof that syphilis was the cause of these lesions is lacking. Strong evidence, however, was furnished in some cases by positive Wassermann reactions of the cerebrospinal fluid. These tests were made independently by Dr. Moody and Dr. Jackson with similar results in every case. Of the fluids tested for the Wassermann reaction from twenty-three bodies, thirteen were positive; from six the cerebrospinal fluid was positive when examined by the Lange test. Of the ten "negative" reports the results in several were noted as "unsatisfactory." It is possible that the percentage of positive reactions would have been larger if the fluids could always have been obtained in the proper condition, and if larger amounts of fluid had been used; 0.25 c.c. was the usual quantity employed. Ellis and Swift⁴² and Hauptmann⁴⁶ agree that the use of a large amount of spinal fluid in tests gives entirely different results than a small amount, especially in the earlier cases, and it is only in such comparatively late manifestations as tabes and dementia paralytica that complement fixation is obtained with amounts as small as 0.05 c.c. Ellis and Swift tested eight cases of what they called "secondary syphilitic meningitis," and with 0.5 c.c. all but one were positive, while with 0.1 c.c. only one out of the eight cases was positive. In cerebrospinal syphilis 33 percent were positive with 0.1 c.c. and 94 percent were positive with 0.5 c.c. of spinal fluid. The results of Hauptmann are parallel, for of forty-four cases of cerebrospinal syphilis forty-two were positive with the larger amount and all were negative with the smaller amount. Nonne and Hauptmann⁴⁷ state that "it is well known that the blood in cerebrospinal syphilis is negative in 20-30 percent of cases, and the spinal fluid used in amounts of 0.2 c.c. is negative in at least 80 percent."

That a negative Wassermann reaction of the spinal fluid does not exclude syphilis of the central nervous system is also well illustrated by three of our cases: two of them (Cases 32 and 48), marked examples of syphilitic meningitis with gummas microscopically and macroscopically, numerous lesions elsewhere in the body and positive Wassermann reactions with the blood, the third (Case 49) with a good Lange reaction for syphilis, a strong Nonne reaction, a positive Wassermann reaction of the blood, and syphilitic lesions elsewhere in the body. Negative Wassermann reactions with small amounts of cerebrospinal fluid,

46. *Deutsch. Ztschr. f. Nervenhe.*, 1914, 52, p. 249.

47. *Neurol. Centrbl.*, 1912, 31, p. 94.

under these conditions, may perhaps be an indication that these were cases which were not instances of dementia paralytica.

The reliability of the Wassermann reaction of fluids taken from dead bodies, except when other infections are also present, hardly needs to be discussed, in spite of the statements, for example, by Thibièrge and Weissenbach.⁴⁸

Attention may be called to the report by Moody, Jackson and Le Count⁴⁹ dealing exclusively with the value of these tests with fluids removed from dead bodies. It contains statistics of such tests made from reports by a number of other investigators, amounting to a total of 2,163 fluids examined with 655 positive reactions, and a table of 190 fluids from dead bodies examined by the authors, 122, or 64 percent, of which gave positive reactions.

There is an interesting discussion by Jacob⁵⁰ of a case in which death followed treatment with salvarsan. On the night before death the fluid gave a distinctly positive reaction which post mortem "continued to develop in a characteristic manner": phase 1 + +, the Lange reaction + + +, the Wassermann reaction +.

In only five cases (Cases 4, 14, 17, 22, and 43) were all anatomical and serological evidences of syphilis lacking, and in none of these were fluids tested for any of the reactions for syphilis. In five other cases without gross anatomic evidence of syphilis, there were indications of disease of the central nervous system. In two cases (Cases 10 and 13), in which the examination was limited to the head, the clinical diagnosis of cerebral syphilis and cerebral hemorrhage and cortical irritation had been made, and histologically gummas were found in the meninges. In the other three instances there were a positive Wassermann reaction of the spinal fluid, a strong Nonne reaction and a positive Lange reaction for syphilis, respectively. In the remaining forty-five cases, lesions were found in the body, which in varying degrees were regarded as syphilitic. These in the order of their frequency are: aortitis, 20 cases; hyperplasia of the spleen, 19; gummas in the liver, 15; gummas in the spleen, 6; gummas in the lung, 3; gummas in the kidney, 2; gummas in the moderator muscle of the right ventricle, 1; scars in the liver or in the capsule of the liver, 5; scars on the leg, 6; scars on the penis, 4; scars in the spleen, 3; scars in the lung, 1; scars on the arm, 1; scars on the scalp, 1; scars on the skin (location not named), 1; other skin

48. Bull. Soc. de méd. lég. de France, Paris, 1911, 8, p. 224; Ann. d'hyg. pub., 1912, 17, p. 81.

49. Tr. Chicago Path. Soc., 1914, 9, p. 129.

50. Ztschr. f. d. ges. Neurol. u. Psych., 1913, 19, p. 188.

lesions resembling those of lues, 1; ulcers of the leg, 2; syphilitic lesions of the bones of the skull, 5; syphilitic osteitis of the long bones and the vertebra, 1; saddle nose, 1; necrosis with perforation of the hard palate, 1; amyloid degeneration in the kidney, spleen or liver, 4; condyloma latum of the rectum, 1; ulcerative condyloma of the ileum, 1; ulcers in the small and large intestine, 1; gummatous enteritis, 1; aneurysm of the arch of the aorta, 2. From the gross changes and the positive reactions with the tests of the cerebrospinal fluid and blood, support is given to the view that the changes in the meninges are syphilitic.

The large number of cases within a relatively short time with syphilitic lesions in the meninges seemed at first surprising, yet the number correlates well with clinical and serological observations. In recent years, as we have stated, attention has been repeatedly drawn to the frequency of involvement of the central nervous system in syphilis.

A summary of the histological changes shows that gummas were found in only ten brains (Brains 1, 2, 3, 10, 13, 21, 23, 32, 48, and 55) after a study of relatively few places; with more extensive histological examination they undoubtedly could be found in others. In selecting sections for photomicrographs, an effort has been made to illustrate the gradual transition between gummas in the meninges and the scars which they leave. Such scars resulting from spontaneous healing or healing after specific treatment retain to a considerable degree the location, shape, and general appearance of their antecedent, the gummas, so that in the anatomic diagnoses the expressions "gummatous meningitis" and "syphilitic meningitis" occur repeatedly when the term "syphilitic scar" would be more correct. These small scars have a characteristic gross appearance; they are well-defined nodules or small, opaque regions of thickening in the pits between convolutions, of a triangular stellate, or other polyhedral form, partaking of the configuration of the location. As they occupy most commonly the arachnoid they might appropriately be called "arachnoid scars." They are found frequently associated with other changes more definitely syphilitic, not only on the same brain, but in the same section; they are found in the meninges of brains with typical gummas and arterial changes said to be characteristic of syphilis, they are present on brains without circumscribed gummas, but with marked cellular infiltration in the meninges, and they also are found when active processes are no longer present, and they represent, as discrete patches of fibrous thickening, all that is left in these places in the meninges of a former acute or subacute inflammation.

Wherever post mortem examinations are made by those experienced in such work and also generally in the literature of pathological anatomy, certain stellate scars of the liver are recognized as scars of syphilis as easily as lesions in the skin due to this disease are recognized by an experienced dermatologist.

There are very few scars in the capsule of the liver that in any way simulate these scars of syphilis. Study of the scars and gradually cicatrizing minute masses of granulation tissue in the meninges has impressed us with the idea that these too, as the scars in the capsule of the liver or the lesions of the aorta, termed nodular arteriosclerosis, may serve for the pathological anatomist as definite indications that at one time a syphilis was incurred.

Naturally, one of the most important reasons for ascribing these scars in the meninges to lues is the demonstration of all stages of development between the minute miliary, or even microscopic, focal masses of granulation tissue, with all the essentials histologically of gummas, and such scars, and also their infrequency unassociated with other evidences of syphilis.

Of perhaps more than ordinary interest is the occurrence of sudden or unexpected death in ten of the fifty-five individuals whose brains were studied. The changes in the meninges were inadequate to explain death. The tissue of the brains showed no noteworthy gross changes. From several of these brains pieces were taken from the interior and examined microscopically. The changes were slight, and a detailed study with various special stains was not made. In searching through medicolegal and other literature, no statement was found that leptomeningitis per se may be the cause of sudden death. Weber,⁵¹ in discussing chronic leptomeningitis, without special reference to that resulting from lues, emphasizes the frequency of acute changes elsewhere in the brain and the spinal cord or the meninges of persons whose death is sudden, and the contributory rôle played by hindrance to the drainage of the cerebrospinal fluid.

Since the information regarding some of these sudden deaths in our series is scanty, the question might be raised whether or not these were possibly cases of dementia paralytica. Our histological examination, as far as it has gone, apparently is not in favor of such a supposition; at least, it does not reveal any features pronounced enough to strongly suggest the diagnosis of general paralysis. We naturally

51. Vrtljsschr. f. gerichtl. Med., 1912, 1, p. 64.

would require a somewhat advanced stage of this disease to seriously consider the liability of sudden death from it. There are some interesting statistics about the causes of death in dementia paralytica, outside of that of marasmus, in an article by Roubinovitch and Paillard.⁵² The authors distinguish between rapid and sudden death; the former occurring from epileptic and apoplectic attacks. According to Arnaud, this is almost the normal mode of death in progressive paralysis. He found that of forty-three paralytics, thirty-four, or 80 percent, died from this cause, while Delmas found such death in forty-seven of one hundred and fifty-three cases. Sudden death is not entirely exceptional. Arnaud found two deaths of this character in forty-three cases, Maraoudou de Montyel found nine in one hundred and four cases, and Delmas four in one hundred and fifty-three cases. From the statistics of these and others, Roubinovitch and Paillard found twenty-five cases of sudden death in five hundred and thirty-eight paralytics, that is 4.6 percent. Sudden death in dementia paralytica occurs chiefly in patients of middle age, in whose previous history there is frequently the record of marked alcoholism. It results from cerebral lesions, most frequently hemorrhage or cardiac disturbances, and without any recognizable cause. Guérin,⁵³ in a thesis, considered sudden deaths in dementia paralytica. In three no post mortem examinations were made; in three others, the cause was cerebral hemorrhage; in one, death followed catheterization; in one it resulted from a fall; and in the ninth, a chronic alcoholic, death occurred suddenly after a meal. In another series of six the post mortem examinations revealed no noteworthy changes. Roubinovitch and Paillard report one instance of sudden death in the initial stage. They conclude that in the majority of cases sudden death cannot be explained. This conclusion probably also applies to those we encountered. More than one factor is likely to play a part. In addition to the evidence of cerebral syphilis there is the history of alcoholism in several cases. Then, also, it seems utterly futile to attempt to draw definite lines between brain syphilis and the initial stages of changes regarded as characteristic of dementia paralytica. Border line cases occur not only clinically, but also from the viewpoint of pathological anatomy.

CASE 1.—Man, aged 28 years, became ill rather suddenly with occasional vomiting, headache, and stupor. There were optic neuritis, rigid neck muscles, and a bilateral Babinski reaction. The clinical diagnosis was cerebrospinal

52. Presse méd., 1909, 17, p. 409.

53. Quoted by Roubinovitch and Paillard: Presse méd., 1909, 17, p. 409.

syphilis, and the treatment consisted of mercurial inunctions. The initial lesion occurred three years before. The symptoms lasted for about six weeks when death occurred.

Anatomical Diagnosis.—The organs of the trunk were entirely negative except for small scars in the apex of the right lung. An exudate around the optic chiasma and the large vessels led to the diagnosis of syphilitic leptomeningitis (Drs. Kuh and Sceleth).

The pia over the convolutions is thin, but the vessels in all the fissures, except those on the occipital poles, are opaque, dull gray, particularly those of the fossae Sylvii. In three or four places on both hemispheres there is a distinct thickening of the pia in the small triangular pits formed by convolutions. There is a pinkish area, 7×15 mm., on the left posterior central convolution, and the pia over this region and over the vessels in the adjoining posterior central fissure is thickened. Under the hand lens the surface is irregular, granular, and slightly elevated. The vessels here stand out as dull, whitish gray, elevated lines. A similar granular appearance of the pia is over the region at the mouth of the right fossa Sylvii. The pia is thickened along the intercerebral fissure, where the very small pacchionian bodies are located. On each frontal pole is an irregular, pinkish-gray plaque of thickening, about 1 cm. in diameter; anteriorly to the plaque on the left side is a round, elevated, warty-looking mass, 3 mm. in diameter, resembling an agglutination of pacchionian bodies. There is a slight depression over the region of the ascending branch of the right fossa Sylvii and the pia-arachnoid here is diffusely thickened and has a granular appearance. On the base is uneven, granular, and rigid thickening of the membrane over the mesial border of the right temporal lobe. The pia over the pons and the anterior part of the medulla oblongata is also somewhat thickened. The vessels are markedly sclerotic. On sectioning the brain two gummas are found. One is in the right insular region in the temporal portion, which is quadrangular in shape, 7 mm. in diameter, approximately 3 mm. deep, and close to it a small hemorrhage, 2 mm. in diameter, is situated in the center of the claustrum. The other gumma, triangular in shape, 4×9 mm., is in the left hemisphere mesial to the hippocampal gyrus.

In sections from the left fossa Sylvii is marked involvement of the pia-arachnoid; endarteritic and periarteritic changes are equally pronounced. The proliferated intima of the Sylvian artery is in some portions three times as wide as the media and adventitia together. The smaller arteries, on the other hand, each have an adventitia three and four times as wide as the media and thickened intima. Other vessels are completely obliterated, and the seat of gummas can often be recognized as such only by the more or less concentric arrangement of its layers. The gummas are in various stages of regressive changes. The lymphocytic infiltration is enormous throughout the entire pia-arachnoid, in the septa, and around the small vessels of the cerebral cortex. There is a huge cellulofibrinous exudate in the outer portion of the membrane. Here and there the pia is firmly adherent to the brain. In sections through the left optic nerve is a dense, round-cell infiltration in the pia covering the nerve. The septa, which extend from the pia down between the nerve bundles, are filled with lymphocytes, while the small vessels in the interfascicular spaces are surrounded with cells.

CASE 2.—Man, aged 65, who died three days after admission to the House of Correction Hospital (Dr. Sceleth). No history.

Anatomical Diagnosis.—Gummatous meningitis; syphilitic ulcers of the small and large intestine; multiple gummas of the liver, spleen and kidney; syphilitic

osteitis; nodular arteriosclerosis, and edema of the lungs. The ulcers of the intestines were studied by Dr. Arkin who demonstrated the *spirocheta pallida* in the lesion.⁵⁴ There is a marked thickening and edema of the pia over the frontal region (Fig. 1) on both sides. In the sulcus frontalis superior, on the right side about 3 cm. anterior to the sulcus frontalis ascendens, the pia is very much thickened and contains a whitish yellow mass, oblong and of the size of a rice grain. In the left sulcus frontalis superior is a mass of thickening, 0.5 cm. in diameter, and of a yellowish color in the center. In both Sylvian fissures the pia is very much thickened in the form of yellowish streaks. In the region of the ramus ascendens of the left Sylvian fissure there are several small yellowish masses of thickening, each about the size of a pinhead. In the middle part of the fissure of Sylvius there is a patch of hard, yellowish thickening, about 1 cm. in diameter, extending from this region down into the gyrus temporalis superior. The large vessels at the base of the brain seem to be normal with the exception of the bifurcation of the basilar artery where there is considerable thickening. (Description by Dr. Rothstein.)

In preparations from the right superior and the median frontal convolutions there are large "endothelial" cells and small lymphocytes rather loosely scattered in the deeper portions of the arachnoid. The arachnoid is greatly thickened directly opposite the sulci, in some of the sections 8.9 mm. The only other noteworthy change is a thickening of the meningeal veins on the side toward the brain; in one large vein the wall towards the brain is three times as thick as the wall towards the surface. In the sections from the left precentral convolution near the fossa Sylvii is a gumma, 6-8 mm. in diameter. The most necrotic place in this gumma is in direct continuity with the huge fibrinous exudate in the sulcus (Fig. 9). This necrotic region, 2-3 mm. in diameter, is rather sharply demarcated and in a small portion of its margin the nuclei are en pallissade. The brain tissue about this region is not extensively infiltrated with cells, but disarranged with small collections of cells here and there, minute regions of necrosis, some small regions of edema, and some larger regions of hemorrhage. The intima of the Sylvian artery is lifted away from the *elastica interna* chiefly by an accumulation of fluid in the meshes, formed by widely separated cells with long branching extensions for a distance of 0.2 mm. Close to the channel in this adventitious layer there are many lymphocytes. Phosphotungstic acid hematoxylin stains bring out the fibrin content of this exudate. The whole process is unlike tuberculous meningitis in the scanty number of cells with nuclei, i. e., the very extensive necrosis, in the relative absence of minute microscopic tubercles or focal lesions, and the absence of giant-cells. In sections from the right insula there is a focal cellular exudate, 1.2 x 1.6 mm. in the very periphery of the arachnoid, and directly beneath it is an oblong necrotic region somewhat larger (Figs. 10 and 11). There are necrotic regions which are not so related to focal cellular exudates; some of them occupy one-fourth of a field 1.8 mm. across. In the deeper parts of the arachnoid and pia there are many lymphocytes rather uniformly distributed. Some of the minute arteries have a greatly swollen and homogeneous adventitia (Fig. 12) and in the deeper parts of the cortex 6-7 mm. from the surface the sheaths of lymphocytes about vessels are wider even than sheaths of those near the surface.

CASE 3.—Woman, 38 years old, at Presbyterian Hospital (Dr. Bassoe), with the complaint of left pelvic pain, nausea and vomiting, backache and leukorrhoea. Panhysterectomy. There was a history of headache with nausea and vomiting

54. Tr. Chicago Path. Soc., 1912, 8, p. 224.

about once a week, lasting two to three days. She had married at 21 years; there were two miscarriages and it was believed she had been infected by her husband. Improvement followed specific treatment at several intervals. In her last attack, during a thunderstorm, while pressing the call button with the left hand, she thought she was struck by lightning; the left arm was cramped; an hour later she was unconscious with twitching of the left side of the face and arm and involuntary urination. Death two weeks later.

Anatomical Diagnosis.—Cerebral syphilis (meningitis); gummatous enteritis; syphilitic scars in the skin; fibrinous and fibrous peritonitis; healed incised wound in the abdomen; hyperplasia of the spleen.

The meninges are very adherent to the calvarium, and the pia and dura have very dense adhesions between them. Along the course of the vessels in the sulci are small patches of thickening. The right hemisphere is one and one-half times larger than the left. There are no gross lesions on the pons, the medulla oblongata, or cerebellum. On the lateral surface of the right hemisphere in the region of the anterior and posterior central convolution above the Sylvian fissure is an irregular gumma, about 5 cm. in diameter, which extends 1-3 cm. into the brain.

A section shows a gumma, 1 mm. long and 6 mm. wide, with the long axis parallel to the surface of the brain. The intimate connection of the gumma with the meningeal changes is clearly seen. From a rounded projection, about 2 mm. in diameter, in the meningeal area the gumma seems to have grown downward and expanded within the brain tissue. Besides this gumma there is no adhesion between the membrane and the cortical surface; the former is easily discernible by a darkly stained, dense infiltration. Microscopic gummas are seen throughout the meninges. The infiltration is marked throughout the pia, in the septa, and around the cortical vessels. There are numerous giant-cells; a few of them are of an unusual size. In another section a smaller gumma, about 3 mm. in diameter, is in the cortex. The meningeal involvement is pronounced. The arterial changes are conspicuous; there is endarteritis with narrowing and complete obliteration of the lumen, thrombus formation, and infiltration of the adventitia. The giant-cells are numerous here.

CASE 4.—Man, 31 years old, in Cook County Hospital in a condition of extreme shock from two gunshot wounds of the chest and abdomen; died fifteen minutes after entrance to the hospital.

The wall of the basilar artery is slightly thickened and with a hand lens appears gelatinous. On the convexity of each hemisphere the sulci are deep and broad over the upper half of the motor regions and over the prefrontal region to about the level of the ascending branch of the fossa Sylvii as well as back to the parieto-occipital fissure. Over this region the pia is more or less thickened, most markedly along the marginal borders. On the left side the pia is transformed into a whitish adherent plaque which covers the gyrus along the marginal border from the incisura sulcus corpus callosum to 5 cm. anteriorly. On the right side opposite this plaque, the pia is in a somewhat similar condition, but the changes are not so marked. In the left fossa Sylvii the pia is slightly thickened. In different places on the convexity in the outer margins of the plaques, also in several of the deep sulci, are small gummas. (Description by Dr. Rothstein.)

Sections from the left, superior, frontal convolution show a lozenge-shaped scar in the outer portion of the arachnoid, fairly opposite the mouth of a sulcus. It stains very poorly. In sections from the right precentral fissure the outer

limit of the arachnoid is rather far away from the exterior of the brain, on an average 1.25 mm., and the sulcus is correspondingly widened. There is a slight infiltration of the arachnoid with cells; most of the appearances suggest edema. In sections from the left precentral fissure there is a lozenge-shaped scar opposite a sulcus, 1.5-0.5 mm.

CASE 5.—Man, 65 years old, found dead. His son stated that his father had always been in good health, with good hearing and eyesight.

The sulci of the brain are wide and deep, especially over the frontal and parietal lobes and the motor regions. The pia is thickened considerably in these regions, with opacities along the vessels and in places where sulci meet. There are several opaque regions of thickening, pin-point to pin-head in size, located within the pia, and others in the broad sulci of both frontal lobes. They are succulent and of a grayish color. Along the median line, the pia is very much thickened in some places. There are some hemorrhages in the pia of the convexity close to the intercerebral fissure, one in the frontal lobe, one in the motor region, and one anterior to the pre-occipital fissure. There are some yellowish plaques over the basilar artery. In the left internal carotid the lumen is occluded by a grayish brown mass. On the cross-section one is able to make out what seems a thrombus. Small pin-head spots of calcified tissue are seen in the pia along the median line in front. (Description by Dr. Rothstein.)

CASE 6.—Colored man, 39 years old, with tuberculosis and delirium tremens in Cook County Hospital (Dr. Leigh). He had convulsions and vomited and refused food at times. He died three and one-half days after admission.

Anatomical Diagnosis.—General hyperplasia of the lymph glands; gummatous meningitis; nodular and encapsulated tubercles or gummas of the right lung; and marked edema and atrophy of the brain. Fatty liver with slight cirrhosis.

The pia is slightly thickened in the central area along the intercerebral fissure of the frontal and parietal lobes of both hemispheres. Triangular grayish white masses of thickening, 2-3 mm. in diameter, are in many recesses of the sulci of the superior and median frontal convolutions of both hemispheres; they are somewhat more conspicuous on the left side. The pia over the vessels of the fossae Sylvii is turbid and slightly thickened. The pia, passing from one gyrus rectus to the other, appears slightly thickened and turbid. On the median portion of the left temporal lobe there is a mass of warty formations resembling pachionian bodies.

In sections from the left inferior frontal fissure, the outer limit of the arachnoid is 1-2 mm. away from the surface of the brain, and beneath the arachnoid down to the pia there are mostly only wide spaces. Opposite the mouth of one sulcus there is a slight thickening in the arachnoid. In sections from the left superior frontal fissure there is a large lozenge-shaped necrotic mass of fibrous tissue squarely opposite the opening of a fissure. It is 2 mm. long and nearly 1 mm. wide. The fibers in this scar stain reddish with Mallory's phosphotungstic-acid hematoxylin.

CASE 7.—Man, 42 years old, Cook County Hospital (Dr. Hall). He had been stuporous for six weeks. He had a history of syphilis twelve years ago. There was bloody rectal discharge; the neck slightly rigid; temperature ranged from 100-102 F. from the time of his entrance until his death three days later. Cerebral thrombosis and syphilis of the brain were the final diagnoses.

Anatomical Diagnosis.—Miliary gummas of the meninges; multiple ulcerative condylomas (gummas?) of the ileum; syphilide of the back; hyperplasia of the

abdominal lymph glands and the spleen; emaciation; marked edema of the brain, and emphysema of the lungs.

Sections through a nodule show this to consist of a vessel deeply situated and covered with a layer of arachnoid which is considerably thickened and infiltrated with scattered lymphocytes. This thickening of the outer portion of the arachnoid is considerable only in the region opposite a large sulcus. There is a diffuse and rather even infiltration with cells throughout the entire pia-arachnoid and also in the loose meshes of the pial septa. There is none about the vessels in the brain tissue. There are several types of cells; those in the septa and along the vessels are almost exclusively small lymphocytes; those in the arachnoid are plasma-cells and large and small lymphocytes. Cell division is rather frequent. There are no focal accumulations of cells. In sections of other nodules the diffuse cellular infiltration is similar to that just described. The nodule corresponds to a lozenge-shaped area of arachnoid tissue, the loose meshes of which are filled with red blood-cells and all kinds of other cells; many plasma-cells, small and large lymphocytes; cells with two nuclei; and large cells resembling endothelioid cells. Red blood corpuscles are also in large number in the subarachnoid spaces beneath the lozenge-shaped area located opposite a sulcus. The large cells are apparently as numerous as the small cells in these sections.

CASE 8.—Woman, 67 years old, who died in the ambulance. She had been sick for years, "something was the matter with her head," and she also complained of her heart and that she could not get air when she walked.

Post mortem shows marked changes like those of syphilis in the large blood vessels, chiefly in the aorta. The left ventricle is considerably hypertrophied. There are "secondarily contracted kidneys."

There is considerable fluid in the meninges, but the membranes are clear. The convolutions of the brain are generally small, the sulci shallow. The vessels at the base have many areas of thickening. Sections from the base of the brain, in which one-half of the circumference of the internal carotid is included, show marked endarteritic changes in the larger vessels. In one artery the proliferated tissue occupies three-fourths of the lumen. The tissue is, in the main, fibrous and compact, the cellular element relatively scanty; it is separated from the old elastica interna by a crescent-shaped area of lacunar spaces which are infiltrated with scattered lymphocytes. Contiguous to this is a triangular region with the base towards the elastica, which is rather densely infiltrated with small round cells. The fibers of the media of the internal carotid are in one place separated and the meshes loosely infiltrated with round cells. The adventitia in the larger vessels are rather evenly and rather densely infiltrated with lymphocytes. There is no endarteritis in the smaller vessels; the media is thickened; there is only a slight infiltration in the adventitia, when it exists at all; there is none about the small vessels in the brain tissue. The outer portion of the arachnoid is thickened. There is a diffuse infiltration in the entire thickness of the membrane.

CASE 9.—Unknown man, 40-45 years old, found dead. No history.

Anatomical Diagnosis.—Miliary gummas of the liver, spleen and meninges (?); chronic mitral endocarditis; fibrous pericarditis; fibrous pleuritis of the apex of the left lung; healed tuberculosis of the apex of the left lung; passive hyperemia of the liver, spleen, kidney, and gastric mucosa; hyperplasia of the spleen, and catarrhal bronchitis.

The gyri are rounded and protruding, leaving between them in many places depressions forming wide entrances to the sulci, but 0.5-2 mm. below the level of the outer surface of the gyrus the sulci are closed tightly. The pia is very slightly and diffusely thickened along some of the larger veins. Over the frontal pole and over the upper motor region it is still partly succulent. The basilar and cerebral arteries are free from sclerosis. Along the sulcus Sylvii the pia has a soft exudate in it. (Description by Dr. Rothstein.) No histological examination.

CASE 10.—Man brought to Cook County Hospital (Dr. Wells) in a comatose condition; he had been sick for three weeks with severe headache and loss of appetite; for the last two weeks chills, fever and sweats, and vomiting. There was marked rigidity of the neck, some tenderness in the splenic region, and the Kernig sign and a Babinsky on the right side as well as a lively ankle clonus were obtained. The cerebrospinal fluid (30 drops per minute) gave positive Noguchi on Nonne reactions. The temperature 102 F. Death, four days after entrance. Diagnosis, cerebral meningitis.

Anatomical Diagnosis.—Syphilitic meningitis. (The examination was limited to the cranial cavity and the brain.) The gyri are to a certain extent flattened and the sulci narrowed over all the regions of the brain except the frontal pole. Over the frontal region anterior to the gyri centrales, the sulci are a little wider than at the other parts of the brain. Over the whole convexity of the brain, with exception of the last 3 cm. of the occipital pole and the region above the fissures of Sylvius, the pia is thickened and whitish, especially along the vessels. Along the fissure of Sylvius on both sides the pia is thickened irregularly. Two centimeters lateral from the left sulcus longitudinalis, about 6 cm. anterior to the gyrus centralis, there is an area of thickening of the pia, 0.5 cm. in diameter, with two nodules, about 1 and 2 mm. in diameter. Just anterior to the lower end of the gyrus centralis anterior and about 3 cm. from the fissure of Sylvius is a milky colored nodule smaller than a pin-head. The pia at the base of the brain is only slightly altered, but on the left side between the N. trigeminus and the N. Oculomotorius is a marked thickening and yellowish-white discoloration. The lining of the lateral ventricles over the corpus caudatum is finely granular.

In microscopic preparations from the fossa Sylvii the outer portion of the arachnoid shows an extensive infiltration of large cells and some small lymphocytes. These are scattered along considerable distances, 1 to 3 mm., suggesting later stages of the formation of local fibrous thickening in the arachnoid. There are no marked alterations in the blood-vessels, except for lymphocytic infiltration in the circumference of a large vein, suggestions of definite, minute, focal lesions.

In sections from the left, median, frontal convolution there is a lozenge-shaped scar (Fig. 8) squarely opposite a sulcus 0.3-0.8 mm. in size, its long axis parallel with the surface of the brain; there is no material in this scar that stains, either cells or fibrils. In the sections are also huge collections of lymphocytes and plasma-cells and in those from the right fossa Sylvii is a slight central necrosis in the focal accumulations.

CASE 11.—Man, 43 years old, Cook County Hospital (Dr. Kerr). The abdomen was very rigid; patient appeared in distress and groaned with pain when moved. On the seventh day he passed into a stuporous condition and responded only after repeated stimulation. Symptoms of paralysis were noticed, the right pupil did not react to light, the left not to distance; the right cheek

was more wrinkled on smiling; the tongue deviated to the paralyzed side; the abdominal reflexes and the left patellar reflex were absent; the Babinsky sign was present on the left side; there were sensory disturbances on the left side; a Nonne test was negative; the urine was passed involuntarily; the pulse and temperature increased. Cerebrospinal syphilis was diagnosed.

Anatomical Diagnosis.—Syphilitic pachymeningitis and leptomeningitis; disseminated syphilitic caries on the inner surfaces of the cranial bones; subdural hemorrhage from luetic necrosis of the right sinus in the dura; marked compression of the brain; large “*plaque jaune*” on the under surface of the brain; bronchopneumonia; decubital sacral gangrene; multiple hemorrhages in the pleura and in the gastric mucosa.

The dura is adherent to the bony wall for a distance of 8 cm. over the right temporal region, where it is thickened and of a light yellowish color. On removal of the dura a large semisolid mass, 8 cm. in diameter by 2.5 cm. thick, is found midway in the temporal region and slightly posterior. It is encapsulated by a thin necrotic membrane. The upper and outer surface of the right cerebral hemisphere is concave with the concavity outward covering an area about 10 cm. in diameter, including the fissure of Rolando. The area is somewhat yellowish in color. Over the superior and anterior parts of the hemispheres the meninges are thickened, especially along the sulci, and adherent in the anterior third of the sagittal fissure. A minute “*plaque jaune*” is present on the under surface of the left temporal lobe. From the anterior surface of the beginning of the foramen magnum is a bony projection pointing backward, 0.75 cm. in height and 1.5 cm. in breadth. Almost the entire posterior fossa presents a granular, worm-eaten appearance. The same condition is present to a less degree in the outer and inferior surface of the middle fossa and to a greater degree in the anterior fossa. The sphenoidal sinus contains a mucopurulent secretion.

CASE 12.—Man, 44 years old, Cook County Hospital (Dr. Bassoe). No history, as patient came in unconscious. There were severe convulsions, occurring every two to five minutes and lasting about thirty seconds, involving all the voluntary muscles; during the convulsion the respiration was almost suspended, causing cyanosis. The pupils were unequal and did not react to light. The patellar reflex was slightly exaggerated; the abdominal and cremasteric reflexes were absent; there was no Babinsky. Cerebrospinal syphilis was diagnosed. The patient died the following day.

Anatomical Diagnosis.—Chronic syphilitic meningitis; bronchopneumonia of the right lower lobe (single region); edema of the meninges over the frontal regions; syphilitic periostitis of the inner table of the calvarium with adhesions between the dura and the bone; syphilitic aortitis; sclerosis of the coronary arteries; chronic hyperplasia of the spleen; hydropericardium.

The meninges are slightly edematous. The dura is adherent in places to the calvarium. The meninges are turbid over the lateral surfaces of the frontal, the parietal, and the temporal lobes. There are whitish regions, irregular in shape, stretched along and just without the meningeal vessels in these places. Their borders are irregular and their long axis, parallel with the vessels, extend for great distances, from the beginning of the Sylvian fissure almost to the longitudinal fissure. There is considerable sclerosis of the basal arteries. The edema of the meninges is most marked over the frontal pole where depressions which are full of a gelatinous material occur. This gelatinous material is held in place by the pia arachnoid.

In sections from the cortex of the left cerebral hemisphere including the posterior central fissure at a point 1 cm. from the fossa Sylvii, there is a marked cellular exudate in the deeper parts of the arachnoid chiefly of small cells.

CASE 13.—Man, 60 years old, Cook County Hospital (Dr. Bassoe). Unconscious; face flushed, pupils equal, eyes looked upward and to left, ptosis of left upper lid, a drooping and drawing back of left angle of mouth, expiratory puffing of left side of the face; the muscles of mastication were strongly contracted; the muscles of the chest showed fibrillar twitching, the right leg a spastic condition, the left leg flaccid paralysis; involuntary bladder and bowel movements. In view of a possible cerebral hemorrhage and cortical irritation, a trephine opening was made in the skull, which revealed no changes other than increased pressure. Death three days later.

There is an opacity over the entire convexity, except the anterior temporal poles, and especially marked over the whole right frontal lobe, where the pia is thickened to form dense opaque plaques over the sulci and rather broad streaks along the vessels. Above the right Sylvian fissure these thickenings have a yellowish tinge. On the right hemisphere there is on the posterior central convolution a flat nodule, 3 mm. in diameter, situated on a mass of opaque thickening. At a short distance from this there is in the Rolandic fissure a group of yellow nodules, each about 1 mm. in diameter; three of these nodules are separate and in a row, a few others fuse to form ridges, about 3 mm. long. There is in addition to this a rounded flat mass, 3 mm. in diameter, with a distinctly raised border on half of its periphery. On the right median frontal convolution near the precentral fissure, there are similar rounded masses, 2-3 mm. in diameter, some with a central depression. In a fissure of the inferior frontal convolution towards the Sylvian fissure there are raised irregular grayish masses, 3-4 mm. in diameter. In a fissure of the anterior central convolution there are two oblong nodules, 2 x 3 mm. Only one nodule can be detected on the left hemisphere near the frontal pole located on a vessel. There is a slight thickening of the arteries at the base of the brain.

In larger arteries (1-2 mm. in diameter) there are no marked alterations; there is no endarteritis in small arteries. About medium-sized veins are triangularly shaped regions with the two longer sides, one against the brain, the other a free surface and the third side against the adventitia. In these the pia is more or less densely infiltrated with cells. There is a perivascular lymphangitis. As the pia stretches across convolutions, the regions beneath are filled with coagulated material. The sections from the right precentral convolution possess a number of unusual features of which the most remarkable perhaps is the presence of many very large cells in an exudate of the pia-arachnoid membranes. They are three to four times the diameter of a lymphocyte; some possess two or more nuclei and many have inclusions. Where they are most numerous they compose two-thirds of the cells present; the remainder are lymphocytes. They are very abundant about a small gumma, 1 mm. in diameter near the bottom of the sulcus. Here the large cells form a well-defined zone on the inside of which a narrow layer of lymphocytes encircles the necrotic center. There are no giant-cells. Smaller gummas, approximately 0.2 mm. in diameter, i. e., as large as large human renal glomeruli, are present in the mouth of the precentral sulcus near the Sylvian fissure. There is also a slight gummatous periarteritis about one of the large vessels in these sections and the large outer surface of this forms one border of a huge serofibrinous exudate. The only other noteworthy alteration is a region of thickening of the outer wall of a vein. In microscopic preparations from the right median frontal convolution near the

Sylvian fissure the cellular exudate is almost entirely of lymphocytes. In these sections as well as in those from the precentral convolution, there are narrow sheaths of lymphocytes about the small blood-vessels in the cortex as deep as the sections go—6-7 mm. In these sections the outer walls of the veins are thickened. Perhaps the most interesting changes are oblong, lozenge- and sector-shaped regions of fibrin in the process of disappearing (Fig. 4). In sections for the pes cerebri the vessels present marked and periarteritic changes. The tendency of the infiltration to be localized is pronounced. The cells are almost all small lymphocytes.

CASE 14.—Died in the ambulance.

Anatomical Diagnosis.—Chronic disseminated syphilitic leptomeningitis; microgyria (occipital); chronic catarrhal gastritis; induration of the lower pole of both epididymides.

On the anterior two-thirds of the convex surface the pia is turbid and thickened. This is most marked on either side of the superior longitudinal fissure for a distance equal to the width of two fingers. There are very definite pits which contain pearly thickenings of the membrane. There is a thickening of the pia about the margin of the cerebellum and in places on the under surface of the brain.

In sections through the right median and left superior frontal convolutions there is a great compact thickening of the arachnoid, which increases from the summit of the convolution towards the sulcus. Opposite the mouth of the sulcus is a scar. There is a great deal of pigment in the entire length of the section in a portion of the membrane beneath the dense outer layer of the arachnoid. The pia is lifted away from the surface of the brain. There is no acute process anywhere. A few scattered lymphocytes and plasma-cells are seen in the arachnoid near vessels.

CASE 15.—Man, hard drinker; died soon after being found on the floor gasping for breath. His general health was apparently good; no history of any disturbance of vision or hearing.

The post mortem showed chronic disseminated leptomeningitis (syphilitic) and slight necrosis of the left parietal bone; hyperplasia of the spleen and of the solitary follicles in the lower ileum.

In the meshes of the pia-arachnoid, especially at the meeting places of the sulci, there are small opaque nodular regions, somewhat thickened and varying in size from a pin-head to 3 mm. These are slightly yellowish and are most marked in the precentral regions of the right and left sides and over the occipital region on the right side. Just anterior to the right and left motor areas and adjacent to the midline, there are two patches of thickening similar to the ones described but larger (about 1 cm. in diameter). There are thirteen or fourteen of these patches. On the surfaces made by sectioning the brain there are no gross changes.

There is an increase in the stroma-like structure of the pia-arachnoid. About the inner half of the entire thickness of the arterial walls is the seat of lime deposition to some degree. In one artery is a clot which possibly is an ante mortem clot. Sections from the interior of the brain, suggested the possible presence of a condition described especially by older writers as "état criblé." There is, indeed, a difference in the appearance of the perivascular spaces of His, the widening of which we are accustomed to contribute to retraction following the process of hardening and fixing. These spaces, in sections from regions below the lateral ventricles and the corpus striatum, are very sharply

delimited towards the brain substance, and frequently strands of tissue (resembling connective tissue with the stain employed), pass from the vessel wall towards the brain surface; in two instances an endothelial lining could be detected. In sections from the left occipital lobe a number of small vessels are occupied, partially or almost entirely, by white cells; some of the polynuclear cells have undergone disintegration.

CASE 16.—Man, found dead.

Anatomical Diagnosis.—Disseminated syphilitic leptomeningitis; edema of the brain (slight); engorgement of the vessels of the pia-arachnoid membranes; gumma in the moderator muscle of the right ventricle; gummas in the liver; syphilitic ulcers of the legs; hyperplasia of the spleen and of the periaortic lymphglands; "saddle-nose"; hypertrophy of the right ventricle with fatty infiltration of the myocardium.

There is an opacity and diffuse thickening of the membrane over the central area of the convexity of the brain. A gelatinous thickening covers the vessels in the two Sylvian fissures. A denser, yellow, triangular thickening is in the horizontal branch of the left Sylvian fissure. There are four gray patches; one in the right median frontal sulcus, one in each Rolandic fissure and the largest one on the frontal pole 1 cm. from the median line.

A slight, scattered cellular exudate is present; the cells are not very numerous. There are no changes about the vessels in the brain substance.

CASE 17.—Man died in great pain enroute to the hospital.

The chief changes in the body, and the only changes which would account for death, were in the brain. All the minute vessels of the pia-arachnoid were greatly engorged with blood. These membranes over the anterior half of the vertex of the cerebrum were cloudy and opaque, with discrete, pearly white, angular regions of thickening in the pit-like meeting points of the sulci of the frontal and parietal lobes. There was also a marked sclerosis of the coronary arteries. The heart muscle was in good condition.

The pia over the anterior half of the cerebrum is thickened. There are round, oval, and stellate patches of a pearly gray color in the sulci or at the junction of sulci. Also, the pia presents a cloudiness in the regions of the sulci. Patches are over the fissure of Rolando, over the posterior central sulcus, and the extreme lateral border of the cerebellum on the right side. On the left side are five patches in the superior frontal sulcus, a small one in the horizontal limb of the Sylvian fissure, and six stellate patches over the posterior central sulcus.

CASE 18.—Unknown man of about 50 years.

Anatomical Diagnosis.—Disseminated fibrous syphilitic leptomeningitis; healed syphilitic necrosis of the hard palate, with perforation. There are three kinds of thickening of the pia-mater. There are stellate thickenings and scars in the parietal regions; the scars are depressed and are usually at the junction of several sulci. The largest scar measures 1 x 0.8 cm. x 1 cm. and is situated 2 cm. from the longitudinal fissure on the left side, in the region of the gyrus centralis. The second type is a membranous thickening most prominent along the longitudinal fissure for a distance of 2.4 cm. The thickest portion of this membrane is 2 mm. The surface of this area is irregular and is studded with pacchionian bodies. The third type is an irregular, whitish thickening of the pia-mater, especially prominent over the frontal gyri and the superior parietal gyri giving a mottled appearance to this region. The pia surrounding the blood-vessels,

especially the middle meningeal and the basilar vessels and vessels composing the circle of Willis, is markedly thickened and whitish in color. This thickening extends laterally about 2 or 3 mm. along the course of the vessels. There is a marked sclerosis of the vessels of the base of the brain.

In microscopic preparations from the right precentral convolution where this joins the Rolandic fissure at the intercerebral fissure are irregular patches of thickening in the peripheral or most outer portion of the pia. Some of these stretch along the pia for distances of from 0.8-1.5 mm. and are 0.2-0.4 mm. in thickness. They contain considerable amounts of elastic tissue. These masses are apparently unrelated to the blood-vessels in their location. There are no changes in the minute arteries or about them in the cortex of the brain. In sections from the right median frontal convolution, there are changes of a similar character but less marked. In these patches of fibrous and elastic tissue there is a slight resemblance to keloids.

CASE 19.—Man, 45 years old, Cook County Hospital (Dr. Grinker), with acute alcoholism; died the day after entrance to hospital.

Anatomical Diagnosis.—Lobar pneumonia; marked edema of the brain; fibrous leptomenigitis (syphilitic); supra-arterial, subepicardial fibroid nodules; gummas of the liver and spleen (capsular); vascularization of the diploë (beginning syphilitic ostitis of the diploë).

There is one region of thickening in the brain 1 cm. to the left of the superior longitudinal fissure in the superior frontal sulcus, about 2-3 mm. in diameter. The shape of the convolutions in the frontal poles of the cerebrum is markedly rounded and the sulci are deep, especially in contrast with the remaining portions of the surface of the brain. (Description by Dr. Rothstein.)

In sections from the region of thickening, the most marked change is the presence of cells loosely scattered in the pia which is thickened where it bridges across the sulci. The thickening of the pia consists of an added number of strands, which in this instance lessen the size and increase the number of the spaces between the blood-vessels and the outermost limits of the pia. These strands stain slightly bluish with pinkish margins with phosphotungstic-acid hematoxylin. The region is 0.2 mm. wide and contains seven to eleven of these strands, with narrow spaces between them. The thickening, therefore, is a scar; this is of great interest in view of the gross appearances. In other places the distance between the outer limits of the pia and the outer surface of the brain is just as great, but only three to six strands of fibrous tissue separate the two in some places.

CASE 20.—Man brought to Cook County Hospital in coma. The reflexes normal; a lumbar puncture proved the spinal fluid to be under pressure, 160 drops per minute. Death occurred the next day. The diagnosis was syphilitic meningitis.

Anatomical Diagnosis.—Bilateral bronchopneumonia and fibrous pleuritis; hyperplasia of the spleen, the retroperitoneal lymphglands, and the solitary follicles of the intestines; diffuse syphilitic leptomenigitis; gummas (?) of the liver; petechial hemorrhages in the mucosa of the stomach; fibrous epicardial patches; fibrous adhesions between the liver and the tissues in front of the right kidney.

A milky appearance of the thickened pia is noticeable over the superior two-thirds of the frontal and parietal lobes beside the longitudinal fissure. Over the deep recesses between sulci the infiltration forms triangular and stellate sheets. Pial thickening is also found over the anterior part of the Sylvian

fissures and the anterior pole of the temporal lobes. Six nodules, close together (a larger oblong one 3 mm. long, the smaller 1 mm. in diameter) are situated on the left anterior frontal lobe, 18 mm. from the longitudinal sinus close over engorged blood-vessels. A smaller oblong irregular nodule, 1 x 2 mm. in diameter, is in the median frontal fissure, about 2.5 cm. from the group just described.

In sections from the right frontal lobe there is a great thickening of the arachnoid which is made up almost entirely of fibrous tissue. The veins have somewhat thickened walls; in some of them the coat has an almost homogeneous appearance. In sections from the right fossa Sylvii the meninges are of a great width. There are scattered throughout them a great many cells, small round cells, plasma-cells some of which have oval nuclei. The cellular element is very pronounced in sections from another region of the Sylvian fossa.

CASE 21.—Man, 47 years old, brought to Cook County Hospital in stupor. Mitral regurgitation was diagnosed. Death eight hours after admission.

Anatomical Diagnosis.—Marked sclerosis of the coronary arteries with calcareous obliteration of the descending branch of the right; fibrous myocarditis; marked fatty degeneration and hypertrophy of the heart; anasarca ascites; bilateral hydrothorax (slight); ancient scar on the penis; ancient syphilitic scars and crusted lesions on both legs; cirrhosis of the liver (syphilitic?); nodular sclerosis of the aorta; gummatous depression in the motor area of the right hemisphere; ancient scar on the anterior surface of the liver; sclerosis of the vessels at the base of the brain; slight atrophy of the brain.

The meshes of the pia-arachnoid are filled with fluid. The sulci are wide and gaping, especially over the vertex. There is a depression in the middle of the right motor region, about 1 cm. across; the pia over this region is thin and movable. There is a rather marked sclerosis of the vessels at the base of the brain.

In sections through the region of thickening in the left median frontal convolution there is a scattered infiltration of lymphocytes and plasma-cells in the pia. There is no fibrin or other evidence of any acute inflammation. In sections through the right fossa Sylvii there is a marked endarteritis, the lumen of the artery being occluded for a large part. There are regions of infiltration in the adventitia. The arachnoid is not thickened to any extent, but lymphocytes and plasma-cells are scattered throughout. The small vessels in the cortex have thickened walls. In preparations from the right anterior central convolution this infiltration is dense and has a distinct tendency to focalize. There is a marked endarteritis and a slight periarteritis of the vessels in the meninges, and a slight infiltration about the vessels in the brain tissue.

CASE 22.—Man, Cook County Hospital (Dr. Harpole). One week before entrance he had had an attack of illness with unconsciousness, fever, and chill, since that time he could not talk, was helpless and had to be fed. The spinal fluid gave a negative Noguchi reaction. Death in five days. Diagnosis, cerebral meningitis.

Anatomical Diagnosis.—Disseminated fibrous leptomeningitis; engorgement of the vessels of the pia-arachnoid; edema of the brain; catarrhal otitis media. (Examination was limited to the head.) There is considerable fluid in the meshes of the pia-arachnoid over the vertex. The cerebrospinal fluid is clear, colorless, and increased in amount. The minute vessels of the pia-arachnoid are engorged and very tortuous. The vessels at the base of the brain are not markedly altered. In the pia-arachnoid there are numerous disseminated patches of

thickening, usually angular in shape, 1-4 mm. in their largest dimensions, and located in the pits formed by the meeting points of sulci in the vertex, parietal and frontal lobes. There are no such changes on the base of the brain.

In sections through the plaque on the right superior frontal convolution joining the precentral convolution, there is a very dense scar-like tissue in the arachnoid, measuring 3.3 mm. in thickness. The pia is densely thickened with a similar tissue. This is especially true in sections taken from the right median frontal convolution. In addition to this fibrous tissue, there is a considerable cell content which is not that of an inflammatory process as ordinarily understood. About one-half of the cells are lymphocytes and the remainder are large cells with cytoplasm which is yellowish brown from pigment or possibly fat (myelin?). In some of the cells, the cytoplasm is very coarsely granular. These cells are found chiefly about the arteries opposite the mouths of sulci. They are numerous enough so that in fields with the immersion lens (0.2 mm. in diameter) there are approximately one hundred, on an average, including both kinds of cells.

CASE 23.—Man, 54 years old, restless and noisy, with scars and bruises on the arms and legs, brought to Cook County Detention Hospital. No previous history.

Anatomical Diagnosis.—Right serofibrinous pleuritis, fibrinous pericarditis; compression atelectasis of the right lung; chronic nephritis ("small granular kidney"); hypertrophy of the heart; stellate scars in the capsule of the liver and spleen.

The cerebrospinal fluid gave a positive Wassermann. There is a slight diffuse thickening of the pia over the anterior three-fourths of the convexity, including the two Sylvian fissures. The sulci and vessels are covered with a grayish film. On the base, the pia is thickened over the optic chiasma and the nerve roots and vessels in the neighboring area.

In sections from the optic chiasm and the right fossa Sylvii, there are large numbers of cells in the pia-arachnoid, and in places these cells are clustered together. In sections from the left precentral convolution, there is a thickening of the outer layer of the arachnoid which in one place, opposite the fissure, is almost transformed into scar tissue. There is an obvious tendency of the cells to focalize. The collections are small. They are especially numerous in that part of the pia which is close to the surface of the brain. A few collections are also in the loose meshes of the inner portion of the arachnoid. The larger vessels in the membrane are normal, but the majority of the small vessels in the cortex are rather evenly and densely infiltrated. In sections from the right side of the pons there is the same tendency to focalized cell grouping.

CASE 24.—Man, 59 years old, admitted to House of Correction in a serious condition. The pulse and the heart action were very feeble, temperature was 2 F. subnormal, and the body was covered with scratches. On the second day the pulse was good and the temperature normal. Ten days later, the temperature again became subnormal, the pulse weak and slow, and the respiration slow. Death on the same day. Diagnosis, alcoholism and carcinoma of the stomach.

Anatomical Diagnosis.—Chronic, disseminated, syphilitic leptomeningitis; gumma of the liver; condyloma latum of the rectum; bronchopneumonia of the right lower lobe; chronic catarrhal bronchitis; edema of the brain; carcinoma of the stomach; chronic diffuse nephritis with secondary atrophy of the kidneys.

On the convexity of the brain the pia has a general gelatinous appearance and is seen as filmy sheets in sulci flattening them out, especially over the upper

half of the frontal and parietal lobes, and more markedly over the left hemisphere. Besides this general opacity there are greenish yellow nodular and flat thickenings. On the right hemisphere are two slightly elevated ridges, one 2 mm., the other about 5 mm. long, in the middle portion of the paracentral fissure; a nodular branched ridge, 5-6 mm. long, 1 mm. high, following the course of the vessels in the parieto-occipital fissure, about 15 mm. from the *fissura longitudinalis*. There is a nodular elevation in its course. This yellowish thickening is continued in the sulcus for 1 cm. as a small nodular ridge and as a triangular, deeply situated area, 2 x 3 mm. On the left hemisphere the focal lesions are: a forked yellowish green linear elevation, the limbs 5 and 6 mm. long, in a deep sulcus of the anterior precentral fissure, about midway between the Sylvian fissure and the *sinus longitudinalis*, and a narrow ridge, 5 mm. long, running along a slender vessel across the middle third of the posterior part of the median frontal convolution to a sulcus with a heavier nodular branched ridge.

Microscopic sections show that about the beginning of the left middle cerebral artery the pia is considerably thickened. On the side of the vessel towards the brain is a mass of fibrous tissue 0.6 mm. wide, loosely arranged. On the outside of the vessel just under the pia is a thickening that resembles a scar. There are bundles of longitudinal muscle fibers scattered along outside the vessel as accessory layers. There are no signs of either acute or chronic inflammation, unless the changes described are considered part of an inflammatory process. In microscopic preparations from the left anterior central fissure midway between the median line and the *fossa Sylvii* there is a thickening of the pia which begins on the convolutions, gradually increases and continues across the sulcus. The thickening consists of bands or layers of loose fibrous tissue and the coarseness of the meshwork between them increases towards the brain.

CASE 25.—Unknown man, about 25 years old, found dead.

The spinal fluid gave a positive Wassermann.

The convolutions of the frontal lobe and the anterior and posterior central gyri of both hemispheres are rounded and prominent, and some of the fissures and sulci in these regions are wider and deeper than normal. At the points where the superior and median frontal fissures meet the precentral fissure there are, on both hemispheres, recesses of considerable depth and extent. Those extending from the median frontal fissures each contain a group of *pacchionian* bodies. On the left side the recess nearer the median line is rendered opaque from a whitish thickening of the pia measuring approximately 1 mm. in depth; white lines accompany the vessels. A recess between the posterior central and the superior and median parietal convolutions is of a similar appearance. On the right side there is a marked opacity and thickening of the pia, about 1.5 cm. long and 4 mm. wide, in the frontal fissure. Along the intercerebral fissure there is a rather uniform thickening of the pia of the posterior part of the superior frontal, central, and anterior portion of the superior parietal convolutions. The *pacchionian* bodies are coarse. A square white thickening, about 8 mm. wide and possibly 3 mm. deep, is over the left superior frontal convolution. The pia over the frontal lobes is succulent. The right and left middle and posterior cerebral arteries and some of the vessels of the circle of Willis have yellow nodular thickenings.

In sections from the right precentral convolution there is great thickening of the outer portion of the arachnoid which extends as a compact layer over the vault of the convolutions as well as over the fissure; it is thinner towards the summit of the convolution. There are lymphocytes and plasma-cells scattered

loosely throughout the deeper portions of the arachnoid and in the pial septa. There is a great deal of pigment free and some contained within cells in the arachnoid beneath the dense outer layer. The vessels present no changes. In preparations from the left median frontal convolution there is in addition to the changes found in the other sections a lozenge-shaped scar opposite the sulcus.

CASE 26.—Man, 42 years old, Cook County Hospital (Dr. Andrews), with compound fracture of the right leg. Amputation; six days later, death.

The post mortem showed hyperplasia of the spleen; general anemia; syphilitic ulcers of the left leg; nodular syphilitic leptomeningitis; beginning carcinoma of the stomach (gumma?) with hyperplasia of the contiguous lymph nodes; hyperplasia of the retroperitoneal, gastric and periportal lymph nodes.

The changes in the meninges are limited to regions about 13 cm. and 8 cm. broad on each side of the intercerebral fissure and extending forward to the frontal poles of the cerebral hemispheres. There is a milky turbidity of the pia over this entire area and a few thick yellowish plaques, partially covering pachionian bodies, partially covered by them. The sulci are flattened out by the pial thickening. The vessels are accompanied by linear opacities on both sides.

There are alterations of a chronic nature in the microscopic preparations from the left precentral fissure. The outer portion of the arachnoid, opposite the mouth of this fissure, is thickened for a distance of 2.5 mm. and the center, 1.5-2 mm. of this region of thickening, is necrotic or contains very few nuclei which stain. In this region and also scattered loosely throughout the deeper portion of the arachnoid are many cells, a number of which branch out, with brownish pigment granules inside them.

CASE 27.—Man, shot in a saloon. Wassermann test was negative.

There are small regions of thickening and turbidity of the pia in the meeting points of the sulci over the frontal and parietal lobe. The minute pial vessels are engorged with blood and more tortuous than normal. There were also syphilitic scars in the capsule of the liver.

CASE 28.—Man, 40 years old, in House of Correction with delirium tremens. Death three days after entrance.

Anatomical Diagnosis.—Disseminative syphilitic cerebral meningitis; traumatic hemorrhage into the left pericranial tissues; myeloid hyperplasia of the diploë; bronchopneumonia; miliary fibrous nodules of the parietal layer of the pericardium.

The spinal fluid gave a positive Wassermann reaction.

In one place in the left frontal lobe where the sulci meet there is a yellowish angular thickening; likewise in the right parietal lobe. In the left cerebral hemisphere, about 2 cm. in front of the interparietal sulcus, there is a greenish gray thickening in the pia 2.5 cm. long and 4.5 mm. wide. There is a marked thickening of the vessels in the pia about the left Sylvian fissure. No histological examination.

CASE 29.—Man, 42 years old, in Cook County Hospital. He was noisy and violent and unable to answer questions. Physical examination was negative. Death on second day after his entrance.

Anatomical Diagnosis.—Fibrous myocarditis; mitral stenosis; microgyria; edema of the brain with engorgement of the vessels of the pia-arachnoid; slight chronic disseminated fibrous leptomeningitis; petechial hemorrhages in the epi-

cardium; marked passive hyperemia of all the viscera; sclerosis of the aorta and coronaries; stellate scars in the capsule of the liver.

The Wassermann reaction of the cerebrospinal fluid was negative.

The minute vessels of the pia are engorged. There are small regions of opacity in the pia over the vertex at the meeting point of the sulci. They are not numerous or large.

In sections from the posterior ramus of the right fossa Sylvii are a few small collections of cells in the deeper portions of the arachnoid in rather definite collections. They are plasma-cells and lymphocytes, rather more of the latter, and among them there are some large endothelial cells. These regions contain brown granules of pigment and in the cytoplasm of some of the large cells there are many fine granules of yellowish green pigment. In the right median frontal convolution is a scar or thickening in the arachnoid, 3 mm. long and 0.5 mm. wide. The larger part of this is necrotic and the necrotic region is lozenge shaped. It is not squarely over the fissure, but a little to one side and the arachnoid here dips down towards the fissure. This region contains many cells which are long, with their axis parallel to the cortex of the brain, with nuclei which do not stain, heavily loaded with pigment granules, some of them so long that they extend one-fourth of the distance of the field of an immersion lens.

CASE 30.—Man, 60 years old, died suddenly. His aunt stated that he had always complained of his brain and had looked as if he had not been in his right mind. His sight had not been very good and he had not been able to hear for twenty years, but understood the motion of the mouth and lips. He had been a hard drinker.

Anatomical Diagnosis.—Disseminated fibrous leptomeningitis; marked edema of the brain; multiple petechiae in parietal pericardium; slight cirrhosis of the liver; slight sclerosis of the coronary arteries.

The blood gave a positive Wassermann reaction.

Where the pia stretches across the base of the brain and covers the anterior perforated space, it adheres in spots, and at the beginning of the Sylvian fissures it is slightly thickened and opaque. Over the vertex it is very thick and gelatinous from the accumulation of fluid. The fissures are wide and distended with fluid. The pia, especially of the sulci, is opaque with angular gray and grayish-yellow opacities which extend as whitish lines on either side of the blood-vessels.

In sections from the junction of the transverse fissure of the right superior frontal convolution with the surface near the intercerebral fissure and in sections from the right anterior central convolution 2 cm. from the midline, the changes consist of a dense but narrow thickening of the outermost part of the arachnoid, a few collections of blood pigment with a scattering of lymphocytes in the deeper parts of the arachnoid and slight increase in the thickness of the outer coats of the small arterioles. The narrow compact thickening in the peripheral part of the arachnoid begins opposite the middle of one convolution and stretches across to a corresponding point on the adjacent convolution, the maximum thickness being close to 180 and located opposite the sulcus, usually near but not opposite the largest of the arteries. This change tapers off to conditions apparently normal near the middle of the convolutions. Sections made from the two fossae Sylvii show similar changes; marked thickening of the arachnoid and a scattered infiltration with lymphocytes and a few plasma cells. The perivascular lymph-space of a vessel situated between the arachnoid and the pia has a distinct endothelial lining on either side.

CASE 31.—An unknown man entered Cook County Hospital (Dr. Morf) in deep coma. No history. Death day after entrance.

Anatomical Diagnosis.—Fracture of base of skull; disseminated fibrous leptomeningitis; syphilis of the aorta with disseminated regions of atrophy; small aneurism of the arch of the aorta; fibrosis of the left lower lobe with multiple minute abscess—unresolved lobar pneumonia (?); old empyema; chronic arthritis of the right sternoclavicular articulation.

The Wassermann test was unsatisfactory.

There are opaque thickenings in the pia-arachnoid over the upper portion of the frontal lobe extending out laterally to the sulci between the first and second frontal convolutions. They are long and narrow and especially marked in the pits between the convolutions.

In sections from the left median frontal convolution there is a marked thickening of the arachnoid, opposite the outer portion of the middle third of the convolution, measuring 4 mm. in maximum extent. On one side, this thickening begins close to the large vessels in the fissure and ceases, as already stated, near the middle of the convolution. In other sections the maximum thickening is located opposite a fissure and the gradual diminution lasts till the next fissure; in other words, it extends clear across the convolution. The thickenings are due to parallel masses of white fibrous tissue forming a felt-like mesh with its greatest density towards the dura and its loosest arrangement towards the pia. In the deeper portions are scattered lymphocytes, a few plasma-cells and other loose cells, which have large amounts of cytoplasm and are phagocytic.

In sections from the interior of the brain in the frontal lobe the changes detected with ordinary stains are an increase in the number of small vessels with complete obliteration of the perivascular space. About many vessels cells are arranged in rows, one and sometimes two. This is especially conspicuous where the capillaries and small vessels are cut in their longitudinal axis. The nuclei of these cells are round, large and rich in chromatin; the cytoplasm is not discernible.

CASE 32.—Man, 29 years old, in Hospital of the House of Correction, with acute alcoholism and dementia. Died in a fit.

Blood from the heart gave a positive Wassermann reaction; the spinal fluid was negative.

Anatomical Diagnosis.—Bolus of meat impacted in the pharynx, occluding the larynx; scar on the penis; gummas of the liver; hyperplasia of tracheo-bronchial lymphglands and of the solitary lymph follicles of the large bowel; hyperplastic syphilitic meningitis.

On the convexity of the brain the pia is thickened in the form of dull, greenish yellow plaques, overbridging the sulci and plastering them over as with putty. Some of these have poorly defined outlines; others have a distinct borderline, raised and smooth, or finely crenated. Some have smooth, dull, filmlike surfaces, others are glazed.

These plaques are more numerous on the right side. They occupy sulci of the frontal and parietal convolutions and of the Rolandic and Sylvian fissures generally at the location where several fissures converge and where they approximately extend over the peripheral one-third of the corresponding convolutions. They are most conspicuous in the fissures, converging towards the Sylvian fissure. There are also many gummatous nodules of various shapes, sizes, and groupings. One single nodule measures from 2 to 4 mm., with an average of 3 mm. A few are globular, with nearly smooth borders. The majority, however,

are flattened, more or less round, irregular in shape and outline, with raised borders, often with an appearance as if stuck on, some with a central depression. A few of these are solitary. In general they form groups. They are located chiefly within and around the left Sylvian fissure. The dura is adherent to the underlying structures just above the Sylvian fissure. Where it can be raised, thick masses of nodules can be seen on the dura and in the fissure. Several clusters of nodules are on the anterior poles of the temporal lobes and two are on the superior frontal convolution of the left hemisphere.

White or yellowish ridges, formed by coalescent nodules and linear elevations in which the nodule fusion is not apparent, are transitional lesions. In the median frontal convolution a chain of densely set, closely apposed, minute nodules follows the windings of a convolution for 2 cm. In the gyrus lingualis, nodules are strung together in a rosary-like formation, 2 cm. long, which appears as if imbedded in the sulcus. Similar groups are seen in the right Rolandic fissure. With the hand lens, vessel-like, greenish yellow lines are seen on both hemispheres not only separately in the sulci and over the plaques, but also running across convolutions. On the basal surface there are plaque-like regions of thickening. There is a slight diffuse infiltrate over the left temporal lobe. Ten round flat nodules, each 2-3 mm. in diameter, are attached to the pia covering the posterior part of the gyri recti. Four irregular nodules are in a group in the anterior portion of the right olfactory fissure. On the left orbital lobe there is an irregular group of about twenty-five flat, raised nodules at the beginning of the Sylvian fissure; the largest one 3-4 mm. in diameter. One cm. from this there is a group of five nodules, averaging 4 mm. each in diameter. On the right temporal lobe, there are three groups, one made up of nine nodules of the flat type with a central depression, each 3-4 mm. in diameter, in a sulcus of the inferior temporal convolution, with nodular extensions toward the pons and in the opposite direction. Another group of eight similar flat nodules is in a fissure of the median temporal convolution, extending towards the group of nodules and masses of nodular infiltrate on the anterior pole of the right temporal lobe that reaches the Sylvian fissure. On the inner surface of the dura mater of the right hemisphere there are seven collections of nodules; the latter measuring 2-3 mm. in height and 3-4 mm. in diameter. On the portion removed from the left hemisphere there are five thick masses of nodules. In some patches the nodules are distinct from one another; in others they are completely fused.

In sections from the right occipitoparietal fissure, 7 cm. from the median line, the sulcus is bridged across in such a way by inflammatory exudate which is largely necrotic and contains small amounts of fibrin that there is practically no indentation to mark its location. Around the margins of this area is a scattered cellular exudate which is rather compact about the small vessels. The cells are chiefly of the lymphocyte type with a very few polymorphous leukocytes and with many large cells of the endothelioid or large lymphocyte variety. The latter are at greater distances from the vessels and frequently contain other leukocytes, red blood corpuscles, or other inclusions. Still another feature of this exudate are the masses of cells which ensheath the minute arteries still deeper in the sulcus, in other words, perivascular changes resembling minute gummas. In the cortex of the brain about the small blood-vessels, which are relatively numerous and relatively large, are also encircling masses of cells from one to three or four cells in width. In sections from the left of the median frontal convolution 5 cm. from the median line, the triangular region formed between the pia and the sides of the convolutions forming the sulcus is occupied by a cellular exudate, most abundant about the arteries. In sections stained with phosphotungstic-acid

hematoxylin, these regions are the places where fibrin is most abundant; it forms a loose mesh which has shrunk away from the wall. These regions contain no red blood corpuscles and they have very definite boundaries lined with endothelial cells. In the deeper parts of the sulcus and in the triangular region, there are early perivascular gummas characterized by very minute spots of necrosis in the exudate of cells, some of them 200-300 μ in diameter, with a few multinuclear or giant-cells. There are also numerous regions of necrosis in the cortex of the brain, about 1 mm. below the surface, rarely at deeper levels. The distortion of neuroglia and nuclei of ganglion-cells and the presence of a few lymphocytes are all the alterations in places of this sort where the changes are least. Where the changes are more marked the cell exudate is more abundant. The presence in some of them of a few red blood corpuscles suggests their development in connection with the perivascular accumulations of cells which are present in many places and in varying degrees. Some vessels are filled with cells, and in some cases necrosis of occluded vessels with all or part of the surrounding envelope of cells has taken place. Cross sections of still others contain large numbers of red blood corpuscles, indicating an accumulation of blood as a consequence of a closure somewhere else. Between the vessels, the brain tissue is rather diffusely infiltrated with lymphocytes and plasma-cells, that is, their distribution is fairly equal, and among them are many large cells with cytoplasm equal to from four to ten times the nucleus in area. Masses of brain tissue are found here also. Some of the large cells, so far as the details are revealed by several methods of staining, are ganglion-cells of the cortex. In sections from the right posterior central fissure there are large gummas, 2.5 mm. in their largest diameters, and there are also other regions fully as large of necrosis without enveloping cellular exudates and local changes to mark them as gummas in the usual acceptation of this term. In parts of their circumference these regions, in some instances, show varying amounts of cellular exudate. They also contain small masses of fibrin. One of these gummas is located apparently in a vessel which, following occlusion by thrombosis with partial recanalization of the organized thrombus, has become changed by the process of syphilis so that its former wall represents the margin of the necrosis and in its former channel are the lymphoid multinuclear and other cells found usually surrounding gummas.

In addition to changes that have already been described, there is in microscopic preparations from the left fossa Sylvii towards the anterior temporal pole a region 2.5 mm. where the cortex of the brain possesses a great many newly formed blood-vessels or where the previously existing blood-vessels have become greatly dilated and so infiltrated with cells that it resembles granulation tissue at first glance. Around many of the small vessels are sheaths of cells, chiefly lymphocytes and plasma-cells. In some of the vessels, their channels are completely occupied with these cells, and such vessels with all or part of the surrounding envelope of cells may be necrotic. Other vessels contain large numbers of red blood corpuscles, indicating an accumulation of blood as a consequence of a closure somewhere else. Between these vessels, with or without cell aggregations, the brain tissue is rather diffusely infiltrated with lymphocytes and plasma-cells, and among them are many large cells with cytoplasm equal to four to ten times the nucleus in area. Some of the large cells are ganglion-cells of the cortex. Masses resembling cells, but without nuclei, are present and undoubtedly represent fragments of brain tissue.

A number of pieces from different regions of the brain surface were prepared by Noguchi's modification of the Levaditi method for spirochetes; also pieces

from the dura from gummatous regions were prepared by the old Levaditi method. Only in one instance was it possible to demonstrate spirochetes, which were scanty and scattered within the wall of a vein in a broad sulcus.

CASE 33.—Man, 25 years old, in Cook County Hospital (Dr. Preble). Infected with syphilis and gonorrhea three years ago. The present condition was diagnosed as acute parenchymatous nephritis. Death about a month after admission to hospital.

Anatomical Diagnosis.—Multiple gummas of the liver and lungs; marked amyloid changes in the kidneys, with chronic parenchymatous nephritis; "sago spleen" with multiple infarctions; amyloid liver with marked passive hyperemia and cyanotic atrophy; hydroperitoneum and hydrothorax; chronic local fibrous leptomeningitis; compression atelectasis of the right lower lobe and entire left lung.

The blood gave a strongly positive Wassermann reaction.

There is no noticeable thickening of the meninges on the convexity, except a thin, opaque film over the meeting points of sulci. There is a denser, gelatinous thickening, polyhedral, about 5 mm. in diameter, in a sulcus of the right occipital lobe, 1.5 cm. from the intercerebral fissure. On the base, a slight opaque thickening of the pia is seen as it emerges from the right Sylvian fossa.

In microscopic preparations from the right fossa Sylvii and the right occipital lobe are no noteworthy changes.

CASE 34.—Man, 59 years old, who had suffered from pain in the back, headache, pain in the epigastrium, occasional vomiting and constipation, became suddenly unconscious, then regained consciousness, but was unable to speak for two hours and could not move his left arm or leg. He was delirious the following night and died the next day. There was a history of syphilis and gonorrhea thirty years ago. He had been a heavy drinker for many years.

Anatomical Diagnosis.—Atrophic cirrhosis of the liver; marked ascites; general icterus; chronic diffuse nephritis, secondarily contracted kidneys; fibrous myocarditis; marked edema of the brain; bronchopneumonia of the upper portion of the left lower lobe, sclerosis of the basilar artery with marked narrowing; fibrous (luetic ?) leptomeningitis. The blood gave a strongly positive Wassermann reaction.

There is a grayish, opaque film in the sulci and fissures and along the vessels over the entire convexity. Yellowish white, nodular, rounded regions on the turbid pia are in several places. A flat, oblong, white nodule, 4x2 mm., with slightly raised borders and a depressed center, is on the left superior parietal convolution. Another nodule, 2 mm. in diameter, is on the summit of the left median parietal convolution. In the corresponding locality on the right hemisphere, there is a flat, oblong white nodule, 2x2.5 mm.

In sections from three places, the right and left median frontal and the right median parietal convolutions, the changes are alike, most marked in membranes over the right median frontal. They consist of slight thickening of the arachnoid and of the outer coats of both the larger arteries and veins. In such places, there are small masses of yellowish granules resembling blood pigment, some in cells, some apparently without, none in masses larger than might be contained in phagocytes.

CASE 35.—Man, 53 years old, Cook County Hospital (Dr. Conley). No history. Double lobar pneumonia was diagnosed. Death the day after entrance to hospital.

Anatomical Diagnosis.—Lobar pneumonia of the right upper and middle lobes; hypertrophy of the left ventricle; syphilitic meningitis; arteriosclerosis—syphilitic (?); “plaques jaunes” of the brain; calcified plaques adherent to the skull.

The serum gave a strongly positive Wassermann reaction; the spinal fluid, no reaction.

There is a gelatinous appearance of the pia-arachnoid over the entire convexity. Yellow white thickenings, punctate and of larger size, are found in fissures and recesses between convolutions, especially on the left hemisphere. The most marked alterations of this type are in the median frontal fissure in its posterior part, in the posterior portion of the superior frontal, and in the anterior and posterior central fissures. On the right hemisphere such yellowish thickening is found in a recess of the median frontal convolution and in the posterior central fissure. There is a diffuse grayish thickening over all vessels, especially in the Sylvian and Rolandic fissures. On the base of the brain, the pia over the pedunculi cerebri is turbid and somewhat thickened. The walls of the basilar artery and of the vessels of the circle of Willis are thickened.

In sections from the middle cerebral artery are small regions of thickening in that part of the arachnoid closest to the dura. One of these, taken as a fair example, is 300μ long and 100μ wide, the longest dimension parallel to the exterior of the brain. It is located just external to a sulcus, and consists of lymphocytes, rather loosely scattered. Some of the thickenings of this sort contain fewer cells. In another region of this sort is a small obliterated arteriole, and as it diminishes in size in some of the sections of the series, the narrow enveloping zone of lymphocytes makes it resemble a giant-cell. In the pia are many lymphocytes.

CASE 36.—Man, aged 61, with “senility and traumatic arthritis,” and later coma and convulsions.

Anatomical Diagnosis.—Gummas of the liver; syphilitic aortitis; beginning aneurism at the junction of the arch and thoracic aorta; syphilitic meningitis (?); marked edema of the brain; turbid cerebrospinal fluid; bronchopneumonia; sclerosis of the coronary arteries; fibrous myocarditis; left ventricular hypertrophy; “plaques jaunes” of the brain.

The spinal fluid gave a positive Wassermann reaction; the serum, no reaction.

There are five “plaques jaunes” on the under surface of the brain. There is a pit just in front of the lower portion of the frontal convolution on the right side, triangular, each side 1.5 cm. in length and with a thickened opaque pia stretched across it. There are similar areas at the junction of the larger sulci, three and four being present on the sides and vertex of each hemisphere. The pia is otherwise transparent. The smaller vessels are uniformly engorged. There is a large amount of turbid cerebrospinal fluid.

In microscopic preparations made from the right superior frontal convolution, the chief change is the presence of scattered lymphocytes in the meshes of the pia. There are a few plasma-cells. The cytoplasm of many of the larger cells is coarsely granular and some of these granules stain with hematoxylin. In others the appearance resembles fine fat globules. At one place near the periphery of a medium-sized artery there is a considerable collection of lymphocytes in the adventitia. The adventitia of some of the arteries is much thickened on the side toward the brain; this is notably so in the left fossa Sylvii, where about one-fourth to one-third of the circumference of a vein, the flattened long diameter of which is 2.2 mm., has a densely constructed tissue reflected over it from the side next the brain, and thick coating, made up of non-striated muscle fiber. In some of the large arteries there is a slight endarteritis.

CASE 37.—Man, 53 years old, Cook County Hospital (Dr. Bloch), in a stupor. The spinal fluid was under considerable pressure; Noguchi test negative. Diagnosis, cerebrospinal syphilis.

Anatomical Diagnosis.—Bronchopneumonia; gummas of the liver, slight syphilitic aortitis; basilar syphilitic meningitis.

The serum was negative; the spinal fluid gave a positive Wassermann reaction.

The brain weighs 1,300 gm. The convolutions of the frontal lobes are very narrow, averaging 0.7 cm. in width, some being only 0.5 to 0.6 cm. On the convexity the pia is generally transparent and without thickening. There are no gross changes in the vessels at the base. Across the anterior perforated space in the triangle formed by the optic tracts and the corpora mammillaria, the pia presents a yellowish gray opacity resembling exudate.

Sections from the involved area at the base are unsatisfactory, because the membrane is badly torn and incomplete. The deeper portion of the pia is thickened, resembling the compact thickening seen frequently in the outer portion of the arachnoid; it is composed almost entirely of fibrous tissue without nuclei, as is shown very distinctly by sections stained with phosphotungstic-acid hematoxylin. There is considerable blood extravasation; in one region the blood-cells are between the pia and the surface of the brain, and in a sulcus they are in all the loose meshes of the pia and around the vessels. Most of the vessels in the brain tissue and some of those in the membrane are engorged with blood; in several places in the nerve tissue blood is poured into the space surrounding the vessels. There is a considerable amount of pigment in the pia. A cellular infiltrate is not present in these incomplete sections. There is marked increase of the neuroglia along the surface.

CASE 38.—Man, age 57, brought to the hospital in a delirious condition. Diagnosis, mitral insufficiency and alcoholism. Death, the following day.

Anatomical Diagnosis.—Syphilis of the meninges; marked edema of the brain; nodular syphilitic sclerosis of the aorta; hypertrophy of the heart; healed and partially calcified thrombo-ulcerative aortic endocarditis; ossification of the falx cerebri; several small brown scars on the right leg.

A Wassermann test of the blood gave no reaction; bile (?) was present; the reaction with the spinal fluid was "unsatisfactory."

Over the entire convexity, except the posterior pole of the occipital lobe, the pia is thickened especially over the central portion along the intercerebral fissure. Beneath the filmy pial covering which passes over the fissures and sulci are diffuse, as well as circumscribed, yellow regions of thickening, appearing as punctate, nodular and larger rounded masses, 2-3 mm. in diameter. They are in the anterior portion of the superior frontal, the median frontal, the anterior and posterior central, and the median parietal fissures of both hemispheres. There are four distinct oblong, raised, yellow nodules, 1 mm. wide, 2-3 mm. long, in the left posterior central fissure, and smaller irregular white nodules along the same fissure and in a transverse fissure towards the Rolandic fissure. There is a thick gelatinous plaque, 1.5 cm. long and 0.5 cm. wide, on the left pole of the frontal lobe and which extends into the intercerebral fissure. Another is on the same hemisphere extending from the anterior to the posterior central fissure and forming a triangle with the base towards the median fissure. There is a plaque with nodular yellow elevations on the right occipital lobe where it joins the parietal lobe. The brain has a peculiar mottled appearance from the gray pial opacity, the yellow thickening, the hemorrhagic areas, and the dilated tor-

tuous vessels. There is a grayish thickening on the basal portion of the left fossa Sylvii. The pia on the base is otherwise unchanged save for a slight turbidity.

In microscopic preparations from the left frontal lobe, the chief change is a thickening of the pia; within it is a great number of small vessels with rather wide outer coats. In between the small vessels, and to a less extent elsewhere, are scattered cells, some of them huge cells with cytoplasm distended with yellowish brown granules and others apparently containing red blood corpuscles. There is a great deal of slightly yellowish brown granular pigment in the thickened pia, not all contained within cells. Most of these large cells or other scattered single cells resemble the clasmatocytes described by Maximow. There are no changes in the adjacent brain tissue, nor in the intima of the arteries. In other sections from the left median frontal convolution, similar alterations are present; loosely constructed thickening in the pia, thickened adventitial coats to small arteries, and the scattered large cells, many of which are loaded with pigment. In sections through the internal carotid and from the fossa Sylvii are endarteritic changes.

CASE 39.—Man, age 49, brought to the Hospital of the House of Correction, with acute alcoholism. Pneumonia developed. Death.

Anatomical Diagnosis.—Hemorrhagic pancreatitis (of some standing); abscess in the lesser peritoneal cavity; old scars on the penis; nodular syphilitic sclerosis of the aorta; old copper-colored scars on the legs, syphilis of the meninges; atrophic cirrhosis of the liver.

The Wassermann reaction of the serum was positive; the spinal fluid was negative.

All the blood vessels are markedly engorged; the sulci are deep. There is a gelatinous, gray thickening of the pia which on both hemispheres extends to the Rolandic fissure, most marked over the frontal lobes and on both hemispheres with about the same intensity. In a recess of the right median frontal fissure is a greenish yellow thickening beneath the gray, pial film. In the central portion beside the intercerebral fissure is a glossy, yellowish white mass of thickening distinct from the extensive plaques formed by the numerous arachnoid villi. There is only slight turbidity of the pia at the base.

In microscopic preparations from the left median frontal convolution near the precentral fissure and from the right anterior central convolution are very few changes, the chief being an edema. In those from the right anterior central convolution there is a slight thickening in the outer portion of the arachnoid. There are no changes in the arteries. In sections from both places there are many cells, partly large cells, partly lymphocytes. In sections through the right median frontal convolution is a lozenge-shaped scar in the arachnoid squarely opposite the sulcus. There are no acute processes in the pia beneath this scar.

CASE 40.—Man brought to Cook County Hospital in deep coma. Morphin poisoning suspected. Death in seven hours.

Anatomical Diagnosis.—Syphilitic leptomeningitis with miliary gummas; copper-colored scar on the leg; pericarditis with effusion.

The spinal fluid gave no reaction.

There is a milky turbidity of the pia over the fissures and sulci which are shallow and narrow. This turbidity is present over the entire convexity except the posterior pole of the occipital lobe, but is most marked over the motor area, where the broadness of the gyri is also a noticeable feature. There is a marked shallowness of the intercerebral fissure, beginning about 1.5 cm. from the anterior

frontal pole and extending for 5 cm. The depth of the fissure here is not more than 1 cm. A resilient hardness is felt over this portion. There are distinct lines, about 1 mm. wide, or more diffuse bands, which extend from the fissures to the adjacent convolutions. A somewhat triangular, plaque-like thickening of a whitish yellow color, 7 mm.x10 mm., covers the left Rolandic fissure and the posterior central convolution, 2 cm. from the intercerebral fissure, about 2 mm. thick. Three elevated ridges, 3 mm.x1 mm., extend from this plaque laterally. Another smaller mass of thickening, 2 mm., is on the right precentral convolution, 7 mm. from the intercerebral fissure, covered by smooth, glistening pia. Besides this type of lesion there are solitary nodules; a very distinct yellow nodule, 1 mm. in diameter, is in the right superior fissure 5 cm. from the frontal pole. With the hand lens two other nodules of similar size are seen distinctly near the former, and close to a diffuse infiltration of perhaps 2 mm. thickness. Two other nodular thickenings are in one of the left transverse temporal fissures 1 cm. below the fossa Sylvii. A flat, yellow nodule close to a diffuse mass of thickening is in the left median frontal fissure, 3 cm. from the frontal pole. On the right hemisphere are two nodules, dark yellow, covered with glistening pia, one in the posterior part of the medial frontal fissure, 10 mm. from the intercerebral fissure, the other in the precentral fissure, 3 cm. from the median line.

Microscopic preparations from the posterior part of the right median frontal fissure possess no changes except patches of fibrous thickening of the pia-arachnoid which are most marked at the outer end of the sulcus. There is no endarteritis or any infiltration about the vessels in the brain cortex. In sections from the left posterior central convolution there is a scattered infiltration with lymphocytes and plasma-cells in the sulcus and in the pia along the outer surface of the brain. The arachnoid is evenly thickened and consists chiefly of dense fibrous tissue. Opposite the mouth of the sulcus beneath the large vessels of the subarachnoid space towards the brain is a region of thickening which resembles scar tissue.

CASE 41.—Man, 57 years old, in House of Correction for alcoholism. The right eye was widely divergent. The abdominal, cremasteric, and patellar reflexes were absent. Diagnosis, pneumonia. Death three days after entrance.

Anatomical Diagnosis.—Right serofibrinous pleuritis; lobar pneumonia of the right lung; old scar on the penis; syphilitic meningitis; calcified and caseous tuberculosis of the left apex.

The serum gave no reaction.

The gyri are rather small, the fissures over the frontal and parietal regions wide and deep. The pia has a gray, gelatinous appearance over the convexity; the occipital pole is free. A broad, white band of thickening, 3-5 mm. broad, 5.5 cm. long, 1 mm. thick, on the right hemisphere passes from the intercerebral fissure along the posterior central fissure and the portion of convolution joining it. A narrow band, 3 mm. wide, 2 cm. long, passes obliquely from this band backwards across the convolution and along the fissure behind it. A similar band, less conspicuous, 4 mm. broad, 6 cm. long, passes along the left precentral fissure. Many of the vessels in the fissures are bordered with a whitish line of thickening. Two thick, yellowish white masses, about 2 mm. thick, 3-4 mm. wide, 10 mm. long, are on the left hemisphere in a transverse fissure of the superior frontal convolution. A white, circumscribed mass 2 mm. in diameter, covered by a gray pial film is in the right Rolandic, a similar one is in the right precentral fissure.

In microscopic preparations from the right fossa Sylvii there is a considerable infiltration of lymphocytes; the cells are found in all the layers of the pia-

arachnoid. The infiltration is diffuse, but a tendency of the cells to collect in aggregations can be observed. The arachnoid is considerably thickened and the width of the thickening is similar over the vault of the convolutions as over the fissure. The veins have thickened walls. There are no changes in the brain tissue, nor about the cortical vessels. The alterations in sections from the left precentral convolution are similar, except that the infiltration is less marked. In one region of the periphery of the arachnoid is proliferation of the endothelial layer.

CASE 42.—Man, 40 years old, brought to Cook County Hospital wildly delirious, and died next day. Diagnosis, acute alcoholism.

Anatomical Diagnosis.—Marked edema of the brain; gangrenous cystitis, ascending cysto-uretero-pyelo-nephritis; syphilis of the meninges (?).

The spinal fluid gave no reaction to the Wassermann test. There was a positive Nonne and a strongly positive Noguchi butyric acid reaction.

The pia with engorged vessels is slightly thickened over the frontal and parietal regions of the convex surface, somewhat more extensively along the intercerebral fissure over the superior frontal convolution of both hemispheres, but more conspicuously on the left hemisphere, where the vessels in the sulci and fissures, particularly those of the fossa Sylvii, are covered by a whitish gray film. Dense masses of yellowish white thickening are in recesses on both hemispheres. On the left hemisphere a prominent yellow nodule, 2-3 mm. in diameter, is in a recess of the precentral and superior frontal fissures; close to it are two minute nodules, pin-point, on a streak of white infiltrate; 5 mm. more posteriorly there are two flat yellow nodules covered by grayish pia. About the middle of the precentral fissure there is another more indistinct mass of thickening, 2 mm. in diameter. Small nodular, rather indistinct masses, covered by the thickened pia, are also in the superior frontal and the median frontal fissures. Two diffuse white masses, 1 mm. thick, 3-5 mm. wide, are on the superior frontal convolution, 0.5 cm. from the intercerebral fissure, near the pole.

Microscopically, the arachnoid is thickened over the mouth of the sulci. This thickness measures 200μ here, whereas, over the middle of the convolutions, it becomes very thin, measuring only 12μ . In sections from the frontal lobe is a lozenge-shaped scar opposite the sulcus.

CASE 43.—Man, 38 years old, in Hospital of House of Correction. He had successions of epileptiform convulsions. Death on day after entrance.

Anatomical Diagnosis.—Bronchopneumonia; chronic diffuse nephritis; turbidity of the pia-arachnoid. Over the anterior and upper half of the two cerebral hemispheres the pia is diffusely turbid, most marked in the sulci. Here the pia stretches across as a cloudy gray, slightly green membrane, but there are no discrete thickenings resembling gummas. No changes in the base of the brain.

The chief feature in sections from the right superior frontal fissure is the compactness of the arachnoid thickening in which also the larger vessels in the subarachnoid space are enclosed; there is frequently no space between the wall of the vessels and the tissue of the arachnoid. There is a diffuse, not marked, infiltration with lymphocytes throughout the entire thickness of the membranes. Plasma-cells and large lymphocytes are also present. There are no changes in the brain tissue. In sections from the right parietal lobe and the right fossa Sylvii the changes are similar. There is a marked thickening of the arachnoid, which is very compact over the summit of the convolutions and looser opposite the sulcus. There are scattered in the meshes of the subarachnoid space and the pia cells of various types, chiefly cells with a large amount of cytoplasm and nuclei

of an oval, round, or irregular shape, some of them being loaded with a golden yellow pigment; cells with darker stained, round nuclei and little cytoplasm are present, but not in great numbers.

CASE 44.—Man, in Cook County Hospital, died after five days with extensive cancer of face.

Anatomical Diagnosis.—Bronchopneumonia, purulent and gangrenous; extensive carcinoma (rodent ulcer) of the left side of the face; gummatous meningitis; marked syphilitic and senile sclerosis of the aorta; sclerosis of the superior mesenteric and coronary arteries.

Serum and spinal fluid gave no reaction to the Wassermann test.

The pia-arachnoid over the convolutions is not noticeably thickened, but it is opaque over the fissures and many of the vessels in sulci and fissures are lined with streaks of milky white thickening. In the region bordering the intercerebral fissure is a general thickening of the pia on both hemispheres; this is more marked at the vertex of the brain and is more extensive on the left side. The chief change in the membranes consists in well-defined, somewhat raised, grayish white masses of thickening, located in fissures or the meeting-points of sulci. There are two such circumscribed thickenings, each 3 mm. in diameter, on the right hemisphere, one in the superior frontal fissure near the precentral fissure, the other in the posterior central fissure, 2.5 cm. from the median line. Two oblong thickenings, 4 and 5 mm. wide, are in the median frontal and the posterior central fissure, respectively. On the left hemisphere is an oblong thickening, 5 mm. long, 2.5 mm. wide, in the posterior part of the superior frontal fissure; another, 3 mm.x4 mm. in the precentral fissure, 1.5 cm. from the median line; one, 2 mm.x3 mm., in the posterior central fissure, 2 cm. from the median line; and two roundish masses, 3 mm. in diameter, closely apposed, in the median frontal fissure, 1.5 cm. from the precentral fissure.

In microscopic preparations from the right anterior and posterior central convolutions is a long scar in the arachnoid, 4.5 mm. long and 0.9 mm. wide at its widest portion, tapering slightly at the ends and made up of heavy collagenous fibrils as wide as those in the corium of the true skin. These bands are somewhat similar to those in keloids; another similarity to keloids is the presence of narrow channels between the bands lined with endothelial cells, lymph channels, by fibers and channels parallel to the surface of the brain. In the deeper portions of the arachnoid is a scattered cellular exudate, not very abundant. The outer wall of some of the large veins is slightly thickened. In preparations from the right fossa Sylvii there is a scattered infiltration with lymphocytes and some plasma-cells in the lower portions of the membranes. The arachnoid is greatly thickened; this thickening is so dense that it has an almost homogeneous appearance. This is also true of the thickened adventitia of smaller arteries in the cortex. Similar changes are present in sections from the frontal lobe.

CASE 45.—A colored man of middle age, Cook County Hospital, (Dr. Kuh). Diagnosis, cerebral apoplexy. Death on third day after entrance.

Anatomical Diagnosis.—Syphilis of the aorta; thrombosis of the aorta; gummatous meningitis; spontaneous pial hemorrhage; erosion of the brain tissue; acute purulent bronchitis.

The surface of the entire brain is vivid red from a diffuse pial hemorrhage. The fissures on the occipital lobes are marked by broad, black lines, the vessels. Over the central portion of the parietal and frontal lobes a veil-like covering over the entire area gives the vessels in the sulci and fissures a purplish brown appearance. There is a greenish yellow thickening present in all the sulci of the

region bordering the intercerebral fissure from the anterior frontal pole to the posterior central fissure, 3 cm. wide. In the left Rolandic fissure, 2.5 cm. from the median line, are two quadrangular masses, 3 mm. in each dimension; an oblong mass, 5 mm. long, over 1 mm. wide, on the summit of which there is a distinct nodule, 2 mm. in diameter; and a triangular mass, 2 mm. on each side. Yellow lines border the vessels in this area. The corresponding area on the opposite side is similar. There are also distinct small nodules, about 1 mm. in diameter, of deep yellow; one such nodule in the right, four on the left hemisphere in the anterior portion of the superior frontal fissure. A yellowish plaque extends along the intercerebral fissure of the left hemisphere from the anterior central convolution to the calloso-marginal fissure, 2 cm. wide. The pachionian bodies, which are small and very numerous, are over this plaque.

In the microscopic sections from the right superior and median frontal convolutions, the prominent feature is the large amount of blood diffused throughout the meshes and spaces of the subarachnoid space, and assuming the greatest proportions in the region opposite the sulci. The blood effusion does not extend into the septa of the brain surface and is absent or very slight in the deeper portions of the sulci, and there are no changes in or about the vessels in the cortex. The second marked feature in these sections is the thickening of the arachnoid. In those from the superior frontal convolution there is a long, lozenge-shaped scar opposite the slope of the convolution which resembles in every respect that in Figure 6 or 8. In sections from the right median frontal convolution, the thickening involves chiefly the arachnoid proper, but it is also present to some extent in the meshes of the subarachnoid space. In the region opposite each sulcus, the scar-like thickening is focalized and would be lozenge shaped but for the uneven outer surface. There is a comparatively large number of cells in one of these regions of thickening, most of them apparently white cells. In some of the sections the thickening of the subarachnoid tissue is marked and well brought out by the phosphotungstic-acid stain. In a region opposite one of the sulci this thickening recalls the form and structure of the lozenge-shaped scars generally seen in the arachnoid.

CASE 46.—Man, 48 years old. Diagnosis, cerebrospinal syphilis; cerebral hemorrhage.

Anatomical Diagnosis.—Syphilis of the aorta; sclerosis of the coronary arteries of the heart; slight disseminated fibrous leptomeningitis; purulent cystitis; numerous bony excrescences in the lining of the left main bronchus.

The spinal fluid gave a weakly positive Wassermann reaction.

The meningeal vessels are engorged. There is a slight opacity of the entire pia on the convex surface, which is a little more marked over the vessels of the two fossae Sylvii. A whitish thickening is present on both sides along the intercerebral fissure. The pia on the base is also slightly turbid. There are no gross vessel changes.

In preparations from the left precentral convolution there is a localized region of thickening in the pia, 1.0 mm. long and 0.25 mm. wide, a lozenge-shaped scar necrotic in its central two-thirds and with some granules of golden yellow pigment in it, both without and within cells. This scar is not squarely over a sulcus, but is a little on one side. In all the sections examined the sulci are very wide and the arachnoid lifted far away from the brain. There are no changes in the blood-vessels and no cellular exudate.

CASE 47.—Man, 63 years old, unconscious when admitted to Cook County Hospital (Dr. Kuh). No history. There was a coarse nystagmus and the left

eye-ball showed internal strabismus; the mouth was drawn to the left; the right cheek was smoothed out; the tongue could not be protruded; there was a spastic condition of both arms with occasional convulsive twitchings of arms, body and legs. The spinal fluid was under pressure; 200 drops per minute. Diagnosis, cerebral lues. Death the next day.

Anatomical Diagnosis.—Scar on the penis; copper-colored scars on the anterior surfaces of the legs; syphilitic aortitis; syphilitic meningitis; gummas of the liver, spleen and kidney.

The serum gave a positive Wassermann reaction, the spinal fluid a weakly positive.

The pial vessels are engorged. There is a marked pial turbidity over the anterior two-thirds of the convex surface extending to and including the fossae Sylvii. The sulci are narrow and shallow. A greenish yellow thickening is seen in fissures and recesses particularly in the region over the superior and median frontal and the central convolutions, on the left hemisphere also in the parietal region extending to the occipital lobe. Under the hand lens these regions of thickening appear as dotted, nodular and rounding, deep, yellow masses covered by a thick, turbid film. The vessels in this entire region are lined by bluish white borders or partially obscured by a white covering. Three triangular plaques, measuring about 10 x 8 mm., are on the right hemisphere along the inter-cerebral fissure.

In microscopic preparations made from the posterior central convolution 3 cm. from the intercerebral fissure there are no evidences of inflammatory processes. No changes are in the cortex, or in the vessels of the cortex of the brain, or about them.

CASE 48.—Colored man, 31 years old, Cook County Hospital (Dr. Grinker). Diagnosis, cerebrospinal syphilis with disorientation.

Anatomical Diagnosis.—Basilar luetic meningitis; multiple gummas of the liver; multiple small scars of the spleen; emaciation; abscesses of the left wall of the chest and the right seminal vesicle; melanosis of the pia covering the anterior surface of the medulla; healed scars of the left wrist and right forearm.

The Wassermann test was negative with the spinal fluid and weakly positive with the blood from the heart.

The pia arachnoid, which passes from the orbital lobe over the optic chiasma, the anterior perforated space, the tuber cinereum, the corpora mammillaria, over the pons and medulla oblongata, is turbid and greatly thickened, so that all the structures named are obscured, and only the cut ends of nerves and the basilar artery with branches are seen. The thickening is especially marked over the optic chiasma, tuber cinereum, and the corpora mammillaria, and the anterior half of the pons. A collection of numerous small gummas, averaging 1 mm. in diameter, and forming a band 1.5 cm. long and 4 mm. broad, is over the right posterior cerebellar artery. Under the hand lens, about forty flat gummas with central depressions can be counted. A group of seven gummas of similar size are between the right oculo motor nerve and the internal carotid. Nodular elevations are on the right gyri rectus and orbitalis; these and others described in the following text are indicated in Fig. 1; about fourteen are counted; they are 1-2 mm. in diameter; seven are on the right gyrus hippocampi; about twenty faint elevations along the anterior edge of the right cerebellum. About six nodular elevations, 1 mm. in diameter, are dispersed on the left gyri rectus

and orbitalis. The sheaths covering the olfactory bulbs are very thick. The basal part of the pia passing over the fossae Sylvii is somewhat thickened. On the convex surface there is only a slight thickening and turbidity along some vessels.

In microscopic preparations made from the region of the corpora mamillaria and of the left oculo-motorius there is less granulation tissue than the gross description indicates. Actual gummas occur in both situations, but the chief change is the presence of an abundance of a fibrino-cellular exudate. This is 2-3 mm. thick in the sections from the corpora mamillaria and nearly as thick from the oculo-motorius region. The gummas are small and located close to the brain tissue. They are flat; in their long dimensions parallel to the surface of the brain, usually just without an arteriole, an average of 0.3-0.6 mm. in their largest dimensions. The gummas possess relatively large proportions of necrosis and the giant-cells, altho not numerous, are very large in many instances. The fibrin fills a region 1-2 mm. wide very loosely, and here and there in its meshes are masses of necrotic cells. When the cells are not necrotic, they seem to be almost entirely lymphocytes and plasma-cells. In places, the fibrin is gathered in curious whorl-like masses with layer after layer wrapped excentrically around the region enclosed. There are very few changes in the adjacent nerve-tissue revealed by the staining methods employed. Close to the brain, the changes about the veins are limited to an infiltration with plasma-cells and lymphocytes; the changes about the arteries, on the other hand, are in general much more marked. The outer coats are loosely or densely infiltrated with cells, much wider than normal. Here, too, are gummas.

With Noguchi's modification of the Levaditi method for spirochetes one organism was demonstrated with absolute certainty. The organism is deeply stained and is just within the outer portion of the cytoplasm of a nerve-cell near the brain surface, outside of which there is a huge fibrino-cellular exudate.

CASE 49.—Woman, 48 years old, brought to the Cook County Hospital (Dr. Kuh) in a stuporous condition into which she had fallen the day before. Death the following day.

Anatomical Diagnosis.—Extensive left sided empyema; compression atelectasis of the left lung; nodular sclerosis of the aorta; scar on the anterior surface of the left lung, resembling scars of the liver attributed to lues; miliary gummas of the liver; rupial eruption of the skin; numerous small, copper-colored, depressed scars of the legs; syphilis—gummas—of the meninges; thin skull bones; area of thickened bone in the frontal region of the skull; fibrous adhesions between the dura and the skull; brown atrophy of the heart; absence of the yellow color in the cortex of the adrenals.

The blood gave a positive Wassermann reaction, the spinal fluid gave a negative Wassermann, but a good reaction for syphilis to the Lange and to the Nonne tests.

The brain is small. The anterior and posterior central convolutions of the right hemisphere stand out very prominently; they are more vaulted and the sulci and fissures in this area are deeper and wider than usual. The pia over the convexity, except the occipital lobes, is turbid and generally slightly thickened, markedly over the right central area, anterior and posterior to the Rolandic fissure. There are in addition four circumscribed masses of thickening of a gray and greenish white color under cover of the dull, pial film: one in the precentral fissure about 18 mm. from the intercerebral fissure consisting of two

closely apposed nodules with diameters of 2 mm. each; a second one in a sulcus between the anterior central and the median frontal convolution, 2 x 3 mm. in diameter; a smaller nodular mass, 1 mm. in diameter, 5 mm. anterior to this in a transverse fissure; and 1 cm. lateral from this, in the precentral fissure, an oblong nodule, 1 x 2 mm. in diameter. There are no noticeable changes in the pia of the base. The basilar artery has hardened and thickened walls.

Microscopically, the arachnoid is thickened over fissures in the frontal region. Many rather loosely arranged fibers form a thickening here, 1.2 mm. in its maximum dimensions. There are a few lymphocytes and plasma-cells in the deeper portions of the pia. These are not aggregated into clumps, but are widely scattered.

CASE 50.—Man, 40 years old, was brought to the hospital with pneumonia. Death, three days later.

Anatomical Diagnosis.—Lobar pneumonia; chronic thrombo-ulcerative mitral and aortic endocarditis; obliterative pericarditis, syphilis of the meninges; nodular sclerosis of the aorta; bilateral obliterative fibrous pleuritis; slight atrophy of the adrenals; old healed scars on the face and neck.

The blood gave a strongly positive Wassermann reaction.

The precentral fissure on each hemisphere is unusually wide and the vessels running along them are engorged. The fissures of the frontal and central lobes are in general well marked, while those of the parietal and occipital lobes are decidedly narrow and flat. The pia is glistening and not noticeably thickened over the convolutions, but milky and opaque in the fissures and sulci of the anterior two-thirds of the convexity, particularly over the left fossa Sylvii. There is a thickening in the form of a greenish gray streak in a sulcus between the left median frontal and the anterior central convolution. Solitary punctiform thickenings are in the left Sylvian fossa, in the anterior portion of the left median frontal fissure, and in the right posterior central fissure, 4 cm. from the median line. Two plaques, each about 8 mm. in extent and approximately 1 mm. thick, are situated on the superior frontal convolution near the precentral fissure of each hemisphere.

The microscopic changes in sections from the left anterior central fissure are a thickening of the arachnoid, which measures 1.5 mm. in its maximum, and the presence of a few lymphocytes and plasma-cells in the deeper portions of the arachnoid. Usually the lymphocytes and plasma cells are widely scattered. In a few places they are rather closely aggregated about blood-vessels. Similar, but less-marked, changes are in the left Sylvian fossa, that is, changes of a thickening in the arachnoid. The cellular exudate is slightly more abundant here.

CASE 51.—Man, age unknown, brought to hospital in coma, diagnosed as uremic. Died the same day.

Anatomical Diagnosis.—Multiple gummas of the liver and spleen, and old, depressed white scars on the hip.

The spinal fluid gave a positive Wassermann and Lange reactions for syphilis.

The brain, weighing 1,420 gm., is symmetrical in shape, and there are no changes, except for turgescence of the convolutions, narrowing of the sulci, flattening of the exterior of the brain and a little milky opacity of the pia where it stretches across the sulci of the convexity of the frontal lobes. There are no discrete lesions in the pia and the vessels at the base of the brain are unchanged.

There are no noteworthy alterations in the pia-arachnoid in microscopic preparations of the brain cortex made at right angles through the ascending branch of the left Sylvian fissure or through the left Rolandic fissure.

CASE 52.—Man, 75 years old. Cook County Hospital. No history.

Anatomical Diagnosis.—Marked nodular and senile sclerosis of the aorta with atheromatous ulcers; marked sclerosis of the cerebral and coronary arteries; gummas of the meninges; arteriosclerotic atrophy of the kidneys and brain; thrombosis of the left coronary artery; infarction of the myocardium—apex of the left ventricle; myomalacia cords; acute dilatation of the left ventricle—parietal aneurysm; hemorrhagic, purulent, and fibrinous pericarditis; miliary fibroid nodules in the outer layer of the pericardium; “granulation” of the ependymal lining of the ventricle of the brain; opacity in the right crystalline lens.

The spinal fluid gave positive Wassermann and Lange reactions for syphilis.

There is a marked edema of the meshes of the pia arachnoid over the vertex of the brain. The pia is yellowish or slightly opaque in places, but these regions are not numerous, large or sharply outlined, except in the left posterior occipital lobe outwards from the superior longitudinal fissure where there is a pearly yellowish thickening at the bottom of a sulcus at its junction with a shallower one. There is a marked sclerosis of the basilar artery. The pia stretching across the base of the brain is translucent and the middle cerebral artery is thick. The lining of the fourth ventricle is granular.

In sections from the posterior branch of the left fossa Sylvii are no other changes except the endarteritis which produced the whitish patch seen externally. This is a crescent-shaped thickening, from 0.3-0.36 mm. at its widest place and occupying from one-half to two-thirds of the circumference in the various sections examined. The inner part of this patch contains many cells with nuclei, spindle shaped or elongated, which are parallel with the periphery of the channel. The outer part is less regularly arranged, possesses many cholesterol slits with some foreign body giant-cell formation. This patch of endarteritis also contains some fibrin.

CASE 53.—Man, 54 years old, in Cook County Hospital. Diagnosis, cerebral hemorrhage.

Anatomical Diagnosis.—Nodular sclerosis of the aorta; disseminated fibrous (luetic?) leptomeningitis; spontaneous rupture of a branch of the middle meningeal artery; extensive subdural hemorrhage; marked compression of the left hemisphere.

The spinal fluid gave a weakly positive Wassermann.

The dura is intact on removal of the calvarium and the left half of the dura is collapsed. There are no changes in the superior longitudinal sinus. The left hemisphere is flattened by a clot between the dura and the brain which is spread out over the cortex. There is a large amount of cerebrospinal fluid. The clot is firmly adherent to the inner surface of the dura, but when removed the dura is left smooth. The region of greatest thickness of the clot is over the lower motor areas. The anterior three-fourths of the vertex of the brain are covered by a milky opaque pia. This opaque area is not surrounded by areas of thickening, but there is one such region over the anterior pole of the left hemisphere. In places, especially over the right hemisphere, the vessels are much engorged. There are no changes at the base of the brain. In the vertical portion of the Sylvian fissure the pia is apparently broken and here there is a

little, greenish brown, older clot which remains in the sulcus when the brain is removed and when the clot and dura are entirely separated from the brain. There are no changes in the calvarium.

In sections from the left fossa Sylvii the arachnoid is lifted away from the brain for a distance of 2 mm. There is a slight endarteritis and a scanty and uniformly distributed cellular exudate and no other change.

CASE 54.—Man, 19 years old, admitted in coma to Cook County Hospital, and died the following day.

Anatomical Diagnosis.—Syphilitic leptomeningitis and old epicardial hemorrhages.

The blood gave a positive Wassermann and the spinal fluid a positive Lange reaction for syphilis.

Over the vertex of the brain, especially the frontal and parietal lobes, there is a thick, greenish opacity most marked in the sulci. All of the small vessels of the pia are engorged with blood. The cerebrospinal fluid is increased, the brain is wet, and the cortex soft.

The prominent feature in preparations from four regions of the convex surface are the evidences of marked edema, associated with adherence in various places of the inner portion of the membranes to the brain surface. The arachnoid, which is considerably but not uniformly thickened in its outer layer, is lifted far away from the subarachnoid vessels and the pia; the subarachnoid spaces are greatly distended. In sections from the left fossa Sylvii are four lozenge-shaped scars opposite the wide sulcus. Outside of this area the tissue is rich in cells; those of the connective tissue type are apparently in excess to the number of other cells. Similar changes are present in sections from the left Rolandic fissure. There is a great preponderance of the cellular elements over the fibrous tissue. The pia is adherent to the brain surface. There is one lozenge-shaped scar in the arachnoid opposite the sulcus.

CASE 55.—Man, 52 years old, brought to Detention Hospital in a stuporous delirium. Diagnosis, pneumonia with a meningeal complication.

Anatomical Diagnosis.—Lobar pneumonia; chronic ulcerative tuberculosis of the apex of the right lung; caseous nodular tuberculosis of the tracheo-bronchial lymph glands; nodular and miliary tuberculosis of the upper lobes of both lungs; multiple miliary gummas of the brain, liver, and small bowel; gummatous scars of the anterior surface of the liver; syphilitic meningitis.

The spinal fluid gave a positive Wassermann and a positive Lange reaction for syphilis.

On reflecting the temporal muscle and scalp there is a depression in the bone in the anterior temporal fossa, 3 x 2 cm. Beneath the depression in the calvarium there is a region, 0.5 cm. in diameter, where the dura is thickened and yellowish. The brain is not adherent to the dura at this point nor the dura to the calvarium. There is a gelatinous substance which has accumulated in the meshes of the pia-arachnoid. The membranes about the superior longitudinal sinus of the brain are thickened and opaque; also about the fossae Sylvii the pia is not glistening, but is finely granular. On the outside of the hemispheres there are a number of small discrete pin-head-sized gummas in the pia-arachnoid. In the region described as granular, the structures seem to be slightly glued together by an exudate.

In microscopic preparations from the left fossa Sylvii there are numerous gummas in the membranes, mainly in two septa dipping down into the brain

substance. There is considerable necrosis in the gummatous tissue. In one of the two sulci the infiltration and the gummatous processes extend from the meninges into the brain tissue. The veins are the chief location for gummas and infiltration; a larger artery in the sub-arachnoid space is practically left intact. The vessels in the brain tissue are without alterations; the infiltration follows the vessels into the cortex and only for a short distance. In some of the sections smaller arteries in the depth of a sulcus are surrounded with a thick coat of tissue rich in cells. The intima is lifted away from the media here and there and the space is filled with small round cells. In only one section a giant-cell is found. Where the gummatous processes in the meninges are marked, the outer portion of the cortex of the adjacent convolutions is frequently involved; there are areas of infiltration of various dimensions, whether the meninges are separated by a space from the brain surface or are closely adherent to it. In microscopic preparations from other portions of the brain, there are similar, but less marked, processes. In some sections from the left frontal lobe there is only a moderate thickening of the arachnoid.

SUMMARY

It is not assumed that the minute scars in the cerebral leptomeninx in all the instances described were caused by syphilis, for other infections may be responsible for them. Nevertheless, we believe that a review of all the evidence presented will convince others, as it has us, that it will be necessary to exclude syphilis before associating these scars with other infections. They bring further evidence of the truly focal character of the lesions of acquired syphilis and should be given a value equal to lesions in other places, as the aorta or liver, in the final summing-up of the evidences for a syphilitic infection obtained by post mortem examination.

Finally, it seems likely that in many instances when syphilis becomes generalized in the human body, perhaps at the time when there is a macular rash, fever, and perhaps a spirochetemia, that among other places where the organisms locate, proliferate, and produce gummas are the membranes of the vertex as well as the base of the brain.

Like the scars and minute gummas in the very periphery of the hepatic circulation just beneath the capsule, which are so common in the liver, these arachnoid scars show that the arachnoid is one of the relatively few places where the organisms find conditions suitable for a limited multiplication after a generalized spirochetemia.

In conclusion, we wish to call attention to the inadequate character of the evidence that alcohol may produce a fibrous leptomeningitis with discrete focal lesions.

EXPLANATION OF PLATES 1-11

PLATE 1

Fig. 1.—Right hemisphere of Brain 2. The pia-arachnoid is of a bluish gray, veil-like turbidity and looks as tho milk had been poured over it and in running off some remained in the deep parts of the sulci. This appearance with the wide sulci and sharply outlined thickenings (a) are the gross changes most commonly encountered.

PLATE 2

Fig. 2.—A drawing of the region of the Sylvian fissure from Brain 32. (a) Cluster of nodular gummas; (b) Chain of minute gummas—gummatous lymphangitis; (c) Plaques of coalescent gummas—"fibrohyperplastic syphilitic meningitis" (Nonne), "Sclero-gummatous meningitis" (Sézary), etc.

PLATE 3

Fig. 3.—The right side of Brain 32, showing coalescent gummas, and plaques resembling paraffin grossly in color and consistency. The lesion is extensive in the lower end of the precentral sulcus (a) spreading out across the beginning of the horizontal limb of the Sylvian fissure.

PLATE 4

Fig. 4.—This lozenge-shaped arachnoid scar, with fibrin in its center (c), is perhaps the lesion most commonly encountered. It stretches squarely across the mouth of the sulcus. (a) Surface of arachnoid; (b) sulcus; (c) region of fibrin; (d) infiltration of lymphocytes about the blood vessels; (e) part of one of the convolutions bounding the sulcus. At other places in this brain a gummatous periarthritis is present (Fig. 5). Brain 13. $\times 45$.

Fig. 5.—Microscopic gumma near the bottom of a sulcus bordering the precentral (right) convolution; phosphotungstic-acid hematoxylin stain. There is a blood vessel (a) in the center of the gumma, a wide zone of fibrin just outside (b), a zone of necrotic tissue with scattered fibrin threads in it (c), and a lightly stained zone of greatly altered, but not necrotic, brain tissue (d). Brain 13. $\times 19$.

PLATE 5

Fig. 6.—Lozenge-shaped scar across the mouth of a sulcus bordering the right precentral convolution. When the scars are old and well cicatrized they resemble keloids, altho sometimes in the region of pachionian bodies, they are easily differentiated. Brain 4. $\times 45$.

Fig. 7.—Arachnoid scar with gummatous focal accumulation of cells in the sulcus bordering the left median frontal convolution. (a) Lozenge-shaped arachnoid scar with a few cells in it; (b) focal gummatous cellular exudate; (c) infiltration about a vein. Brain 10. $\times 60$.

PLATE 6

Fig. 8.—Arachnoid scar over the mouth of a sulcus limiting the right inferior frontal convolution. After a search in many places gummas were finally found (Fig. 9). Brain 2. $\times 21$.

Fig. 9.—Small gumma in the left precentral convolution near the fossa Sylvii from a section stained with simply hematoxylin and eosin, therefore the fibrin is not black as in Fig. 5. The cellular exudate about the gumma (a) is directly continuous with that in the pia-arachnoid of the sulcus (b); arteria fossae Sylvii (c); marked endo-arteritis and periarthritis. Brain 2. $\times 8$.

PLATE 7

Fig. 10.—Thickened arachnoid with focal lesions over a sulcus in the right insula. At (a) a fresh or recent cellular exudate shown enlarged in Fig. 11; at (b) an arachnoid scar, somewhat necrotic in the center shown enlarged in Fig. 12; at (c) changes about small arterioles and the scattered cellular exudate. All these alterations, it will be noticed, are in the outer part of the arachnoid. Brain 2. $\times 10$.

Fig. 11.—Transitional focal lesion, neither gumma nor scar, shown at (a) in Fig. 12. It resembles a gumma more than any other of the changes. The cells are chiefly small lymphocytes. $\times 40$.

PLATE 8

Fig. 12.—The patches of thickening in the arachnoid which are found so frequently and in the gross examination were termed gummas, were scars, and as the one shown here, often have necrotic centers. Shown at (b) in Fig. 10. $\times 40$.

Fig. 13.—Cellular exudate and changes in the outer walls of small arterioles shown at (c) in Fig. 10. $\times 40$.

PLATE 9

Fig. 14.—Transitional lesion. Focal cellular inflammation about a blood vessel, neither gumma nor scar. Brain 35. $\times 45$.

Fig. 15.—Transitional lesion. Focal inflammation in the arachnoid. Brain 35. $\times 45$.

PLATE 10

Fig. 16.—Chronic changes in the pia about a vein and depressions in the brain in the epicerebral space, possibly from edema. (a) Thickened pia reflected on the vein; (b) perivascular space; (c) epicerebral space. Brain 36. $\times 45$.

Fig. 17.—Focal gummatous cellular exudate (a) in the wall of a vein from the right Sylvian fossa; (b) cellular exudate in the pia-arachnoid meshes. Brain 10. $\times 60$.

PLATE 11

Fig. 18.—Microphotograph of a section from a fibrinopurulent exudate with gummas (Fig. 5). Compare these changes with those about the arterioles in Fig. 13. The section is stained with phosphotungstic-acid hematoxylin, is fibrin black. From the left oculo-motor region. Brain 48. $\times 90$.

Fig. 19.—Gummatous nodular syphilis of the meninges—a rare form of syphilis characterized by a hyperplastic inflammation. Gummas in the occipitoparietal fissure. The gummas occupy the place of blood-vessels. (a) Remains of an artery; (b) portion of the proliferated elastica interna; (c) newly formed vessels in the gumma occupying former arterial lumen. Brain 32. $\times 25$.



PLATE 2

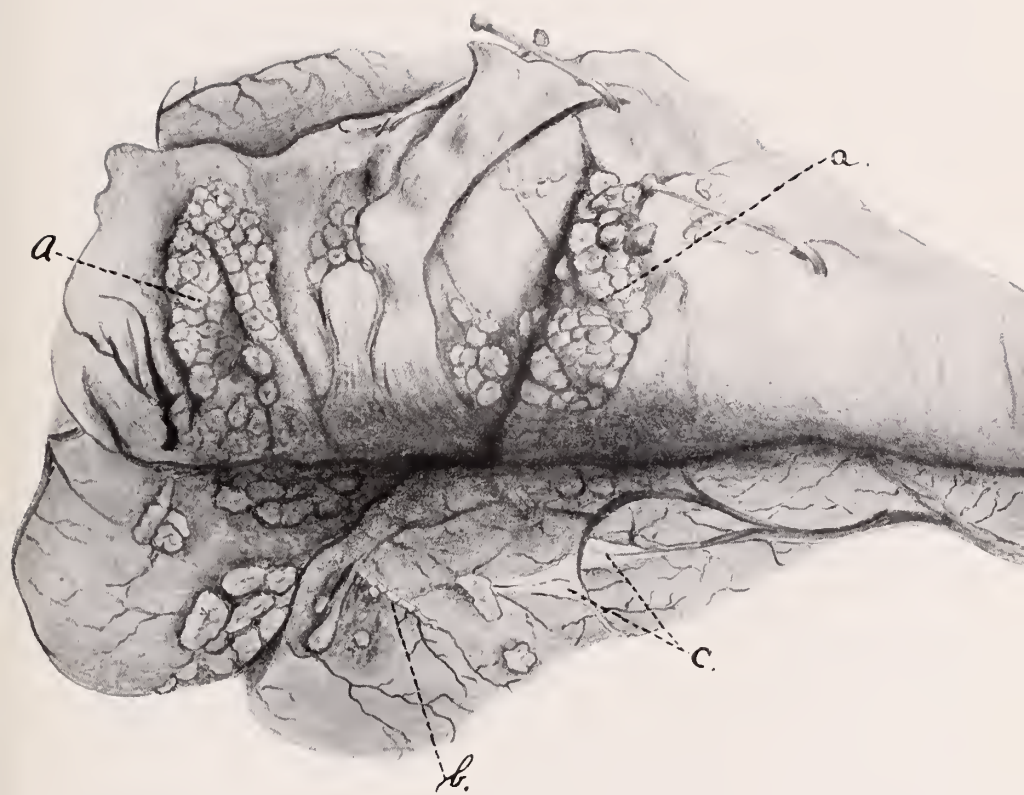


Fig. 2



PLATE 4

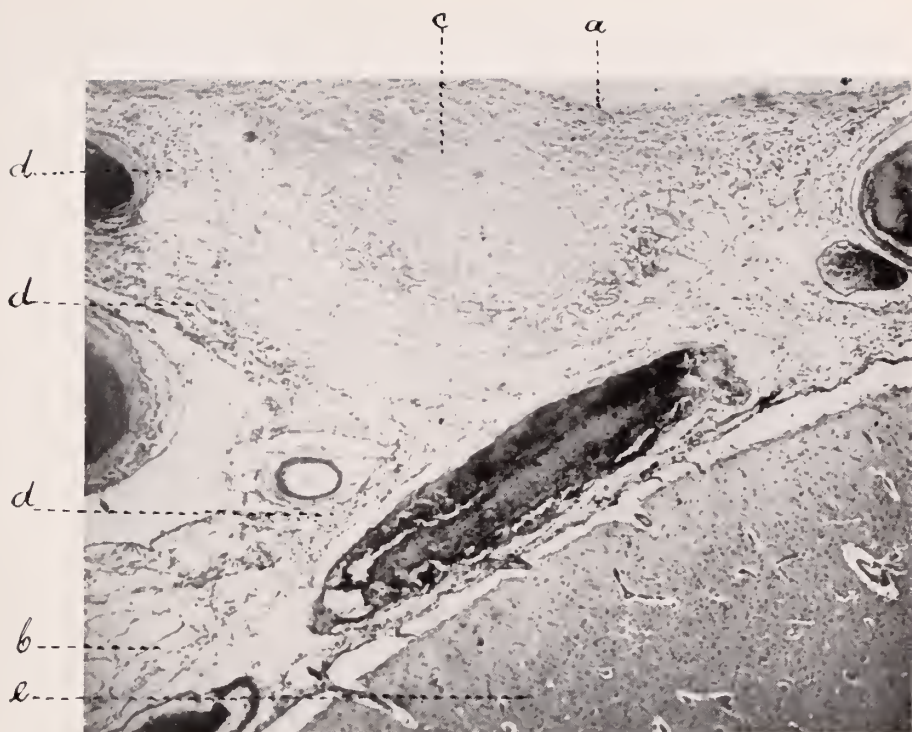


Fig. 4

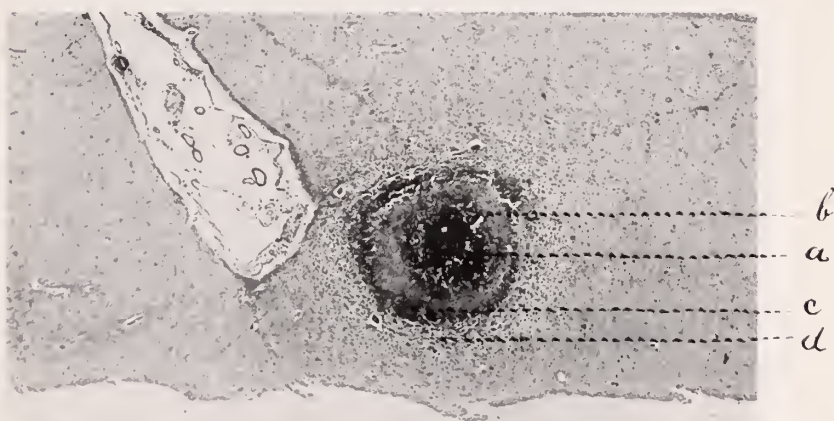


Fig. 5

PLATE 5

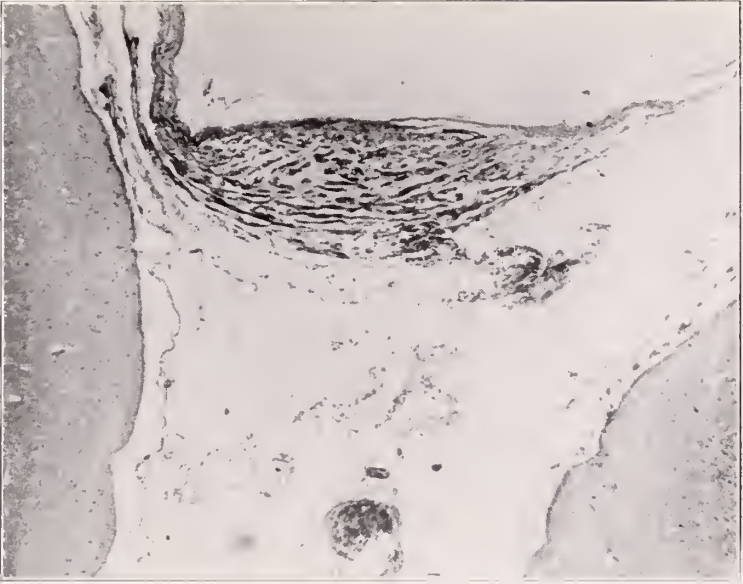


Fig. 6

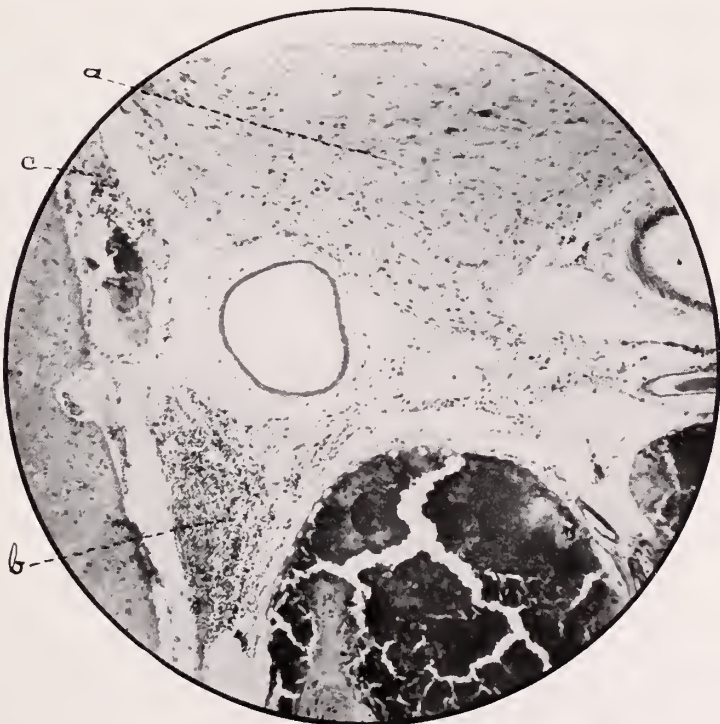


Fig. 7

PLATE 6

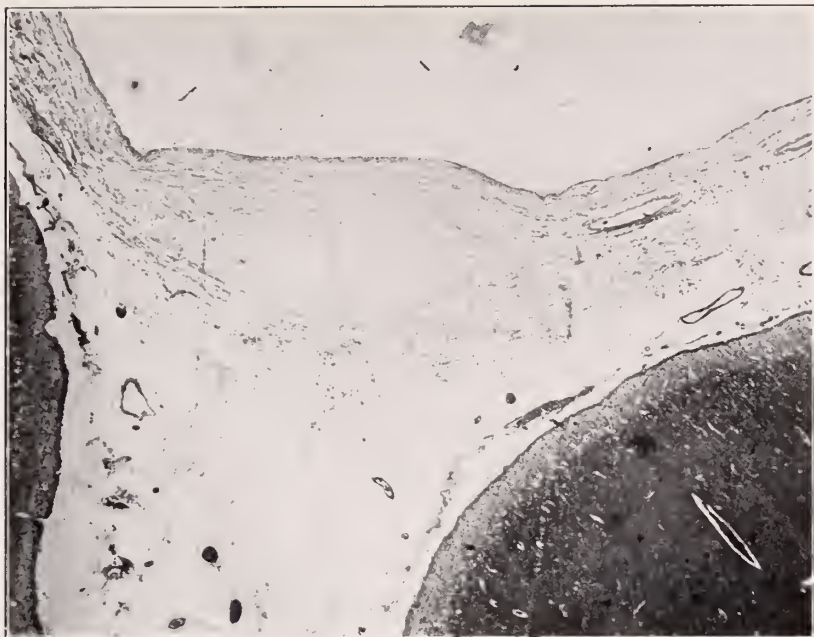


Fig. 8



Fig. 9



Fig. 10



Fig. 11

PLATE 8



Fig. 12

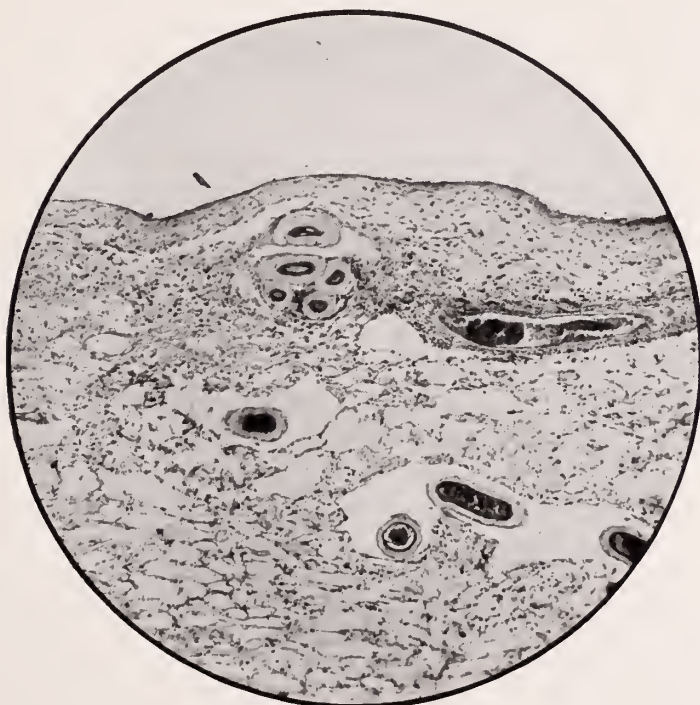


Fig. 13

PLATE 9

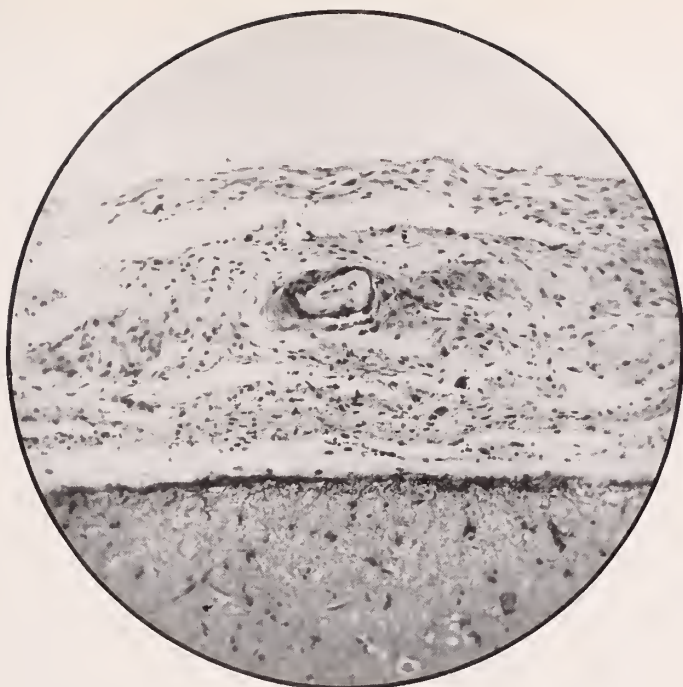


Fig. 14

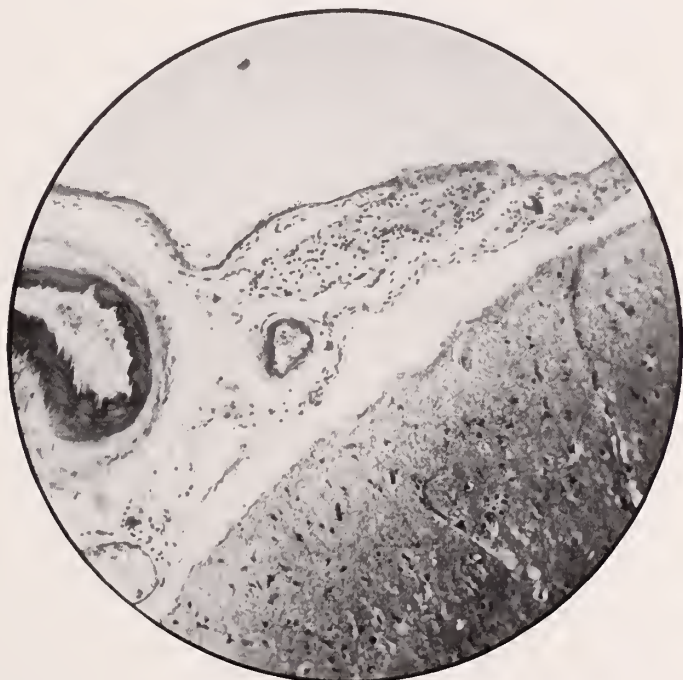


Fig. 15

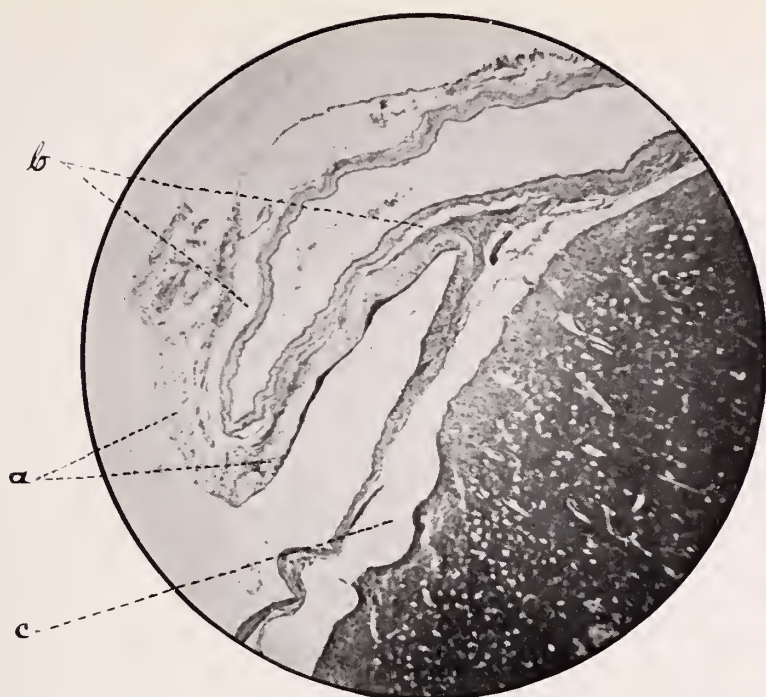


Fig. 16

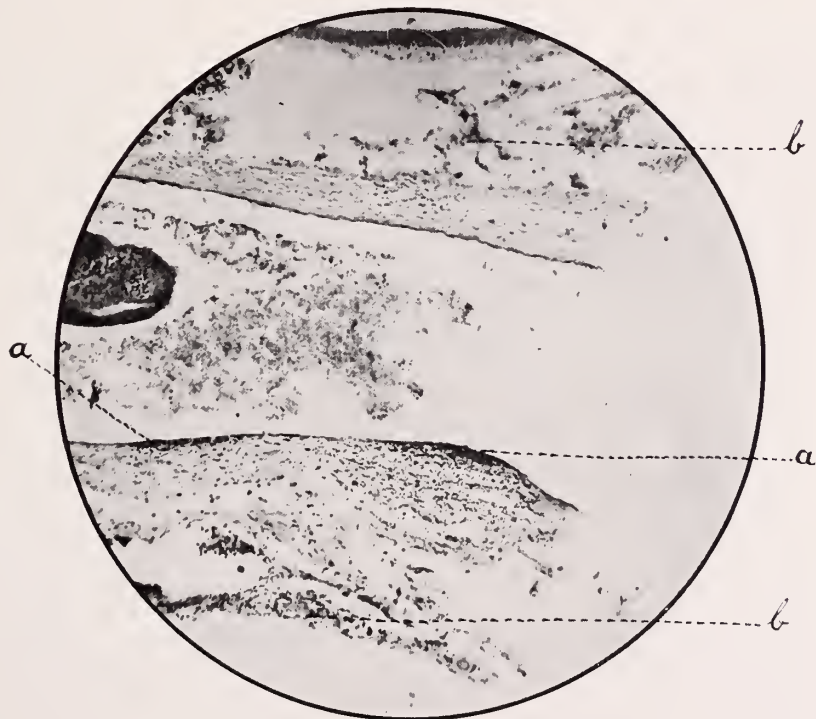


Fig. 17

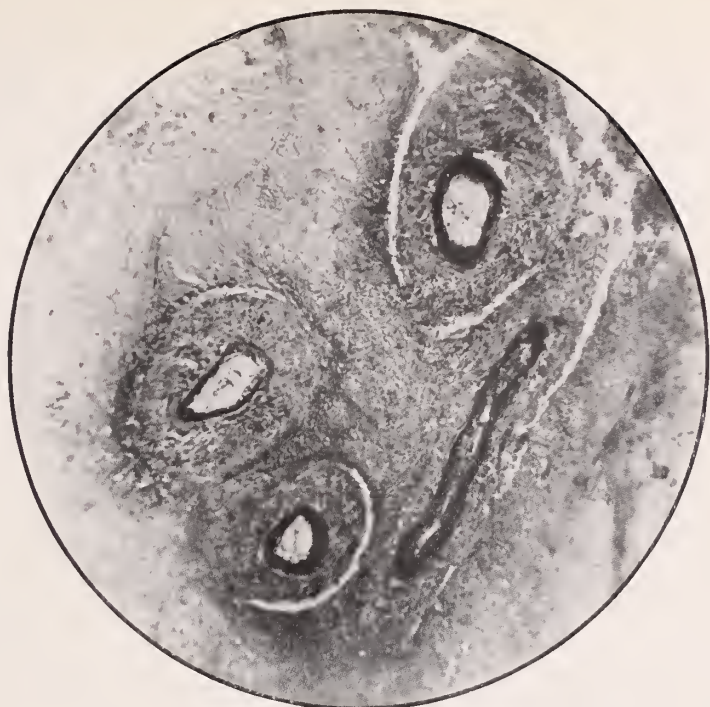


Fig. 18

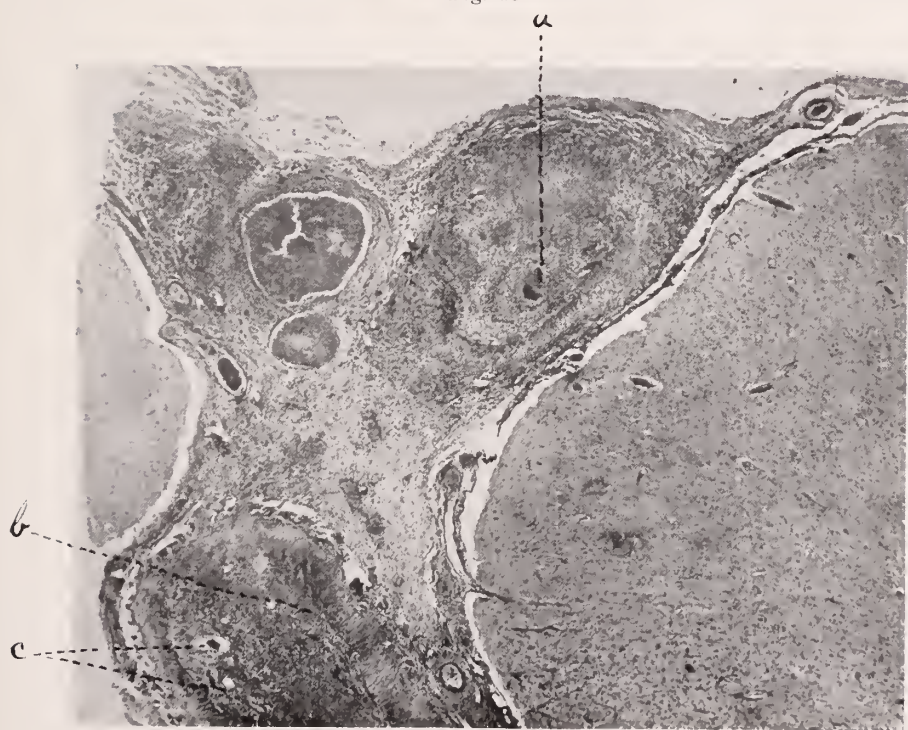
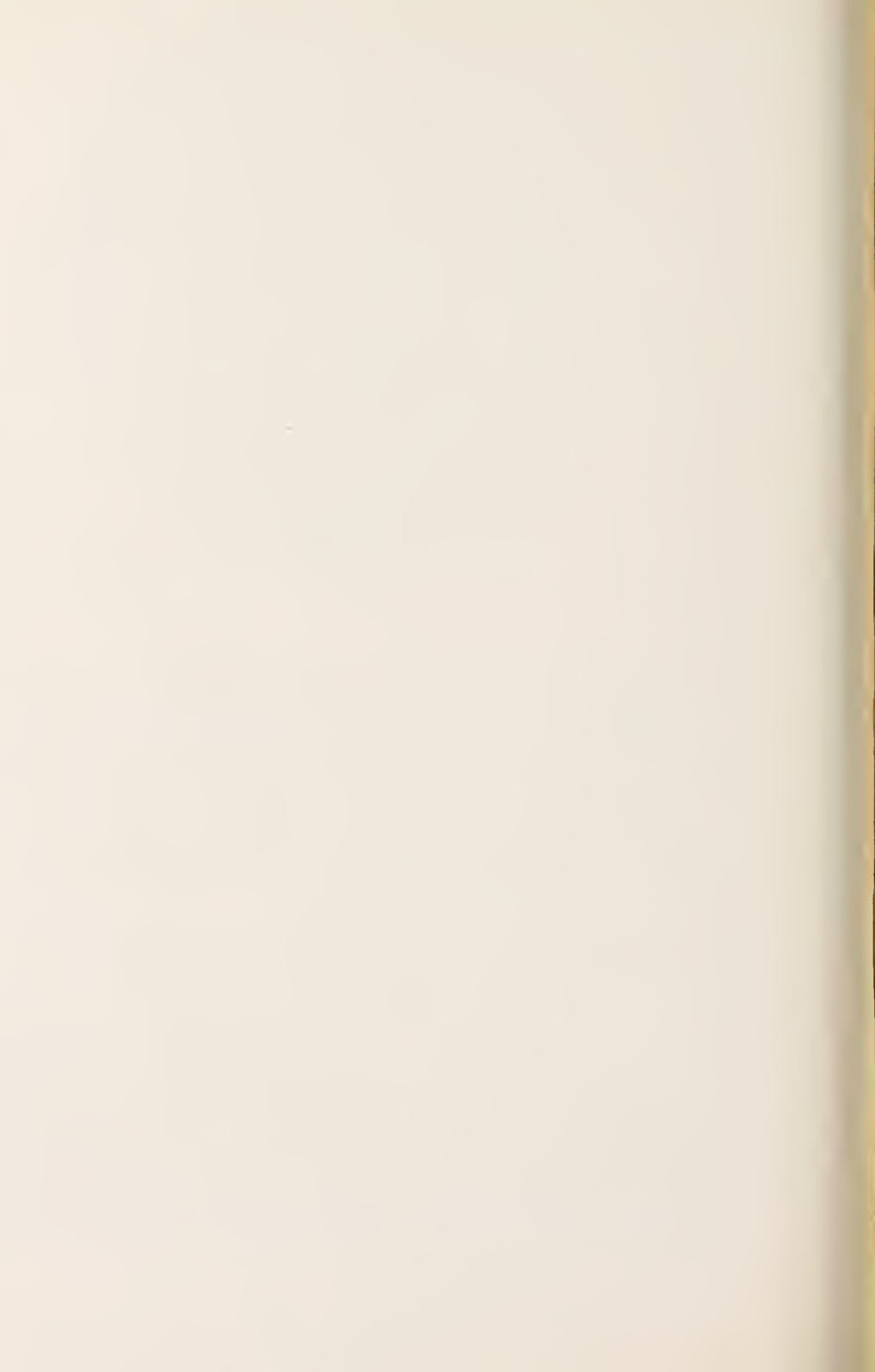


Fig. 19



STUDIES ON THE CULTIVATION OF THE VIRUS OF VACCINIA, III

WITH A NOTE ON THE GLYCERIN RESISTANCE OF VARIOUS
ORGANISMS *

EDNA STEINHARDT AND MARIE GRUND

(From the Research Laboratory, Department of Health, New York City.)

In previous work on the virus of vaccinia, done in conjunction with Drs. Lambert and Israeli,¹ it was found that, in combination with tissue cultures, there was a definite multiplication of the virus with active transfers to the third generation and a resistance of the virus to thirty-four days' incubation at 37 C. We found that living tissue was necessary to the growth of the virus in this method and that the cornea of the rabbit or guinea-pig was more favorable than the heart, liver, or kidney. In the corneal preparations, the epithelium showed an active lateral spreading throughout the clot, forming sheets or groups of cells in the plasma. The cells early showed an accumulation of fat in their cytoplasm, but frequently retained their form for several weeks, even when not transferred to fresh plasma. In these preparations, altho the corneal cells were living and the virus of vaccinia was multiplying, we were unable to find any specific vaccine bodies; only smaller, undifferentiated forms have been seen. These undifferentiated forms have been found in the controls in incubated corneal preparations without the vaccine virus and in the incubated virus corneal preparations. We have observed numerous granules in the incubated preparations, but these have not been sufficiently definite in character to allow us, with the methods employed thus far in our studies, to make any positive statements in regard to them.

There is no growth of the virus of vaccinia in preparations containing cornea killed by freezing, or by hypotonic salt solutions, nor in preparations in which pieces of paraffin are substituted for tissue. The virus is soon rendered inactive in preparations containing plasma and cornea from an immune animal, altho the corneal epithelium may grow very well.

* Received for publication December 3, 1914.

1. Jour. Infect. Dis., 1913, 13, p. 294; Ibid., 1914, 14, p. 87.

Having thus shown multiplication of the virus of vaccinia outside of the body, altho only in conjunction with living tissue preparations, we wish to record at this time results of the purification of the virus for cultural experiments, and culture experiments according to the usual and special bacteriological technic, including a repetition of Fornet's methods. We have also continued our experiments on the resistance of organisms to glycerin.

EXPERIMENTS

Purification of the Virus.—To obtain an active virus, freed from contaminating organisms, several methods have been used. The sterilization with ether, as described by Fornet,² was unsuccessful in our hands, altho tested on a number of viruses taken from both calves and rabbits. We were unable to obtain a virus purified by ether that was not almost entirely inactivated, thus agreeing with the results of Gins.³ The method of sterilization by the use of chloroform vapors, as recommended by Green⁴ and by Nijland,⁵ gave us somewhat more favorable results, but the virus was frequently greatly weakened.

The method which gave us the best results was the one originally used by us in the tissue culture experiments, namely, dialyzation after purification by carbolic acid and glycerin. If the virus was then not entirely free from contaminating organisms, carbolic and glycerin were again added and the dialysis repeated, giving a virus both active and pure. This dialyzed virus was used largely in our present work, altho the ether and chloroform viruses were also tested.

The activity of the virus was always tested by the method of Calmette and Guérin, which consists in inoculations of the freshly shaven skin of a rabbit.

Cultural Experiments.—In experiments on the growth of the virus of vaccinia, Fornet records activity of the virus after numerous transfers on the usual media, agar and broth, kept anaerobically at incubator temperature. The viruses were purified by ether, and the dilution of the original virus by the transfer was very great. We have made numerous attempts with both the calf and the rabbit viruses to repeat Fornet's work, but always with negative results. These negative results agreed with those of Nijland.

2. Berl. klin. Wchnschr., 1913, 50, p. 1864.

3. Ibid., 1914, 51 p. 391.

4. Lancet, 1903, 1, p. 1738.

5. Arch. f. Hyg., 1906, 56, p. 361.

We also have not been able thus far to cultivate the virus of vaccinia by usual or special bacteriological technic. The inoculated media have been kept both aerobically and anaerobically at temperatures varying from 33-37.5 C. The virus was purified usually by one of the three methods given, but we have also used the pure, active, and multiplying virus from incubated virus tissue preparations. The media inoculated were tubes of beef agar, salt free veal agar, glycerin agar, glucose agar, and 1 percent serum (calf or horse) agar; broth, acid, or alkaline with calcium; egg media, Dorset's, solid and non-inspissated; rabbit or guinea-pig plasma and tissue; Noguchi media, with ascitic fluid, horse or calf serum; Hata media; and plates of plain (beef) agar, or in mixture with animal tissues or fluids.

In no instance have we observed any evidence of multiplication of the virus. On solid media the virus remained active longest on agar plates streaked with purified virus; five to eight weeks when kept anaerobically at 33 C. Neither the original agar plate nor transfers to fresh agar plates or to other media gave any indication of growth of the virus. On solid egg and in Noguchi media, the virus has remained active for two to four weeks. The virus rapidly lost its activity when incubated in fluid media containing calcium, or of an alkaline or neutral reaction. In broth, 2-2.8 percent acid to phenolphthalein, the virus remained active a number of weeks and to the second and third transfers. There was however no indication of multiplication, the activity being accounted for by a dilution of the original virus. Similar results were obtained with fluid egg, non-inspissated Dorset's medium, fluid Noguchi, and Hata medium, and also in broth filtered from old tubercle cultures which contained 5 percent glycerin and was 2.8 percent acid.

RESISTANCE OF ORGANISMS TO GLYCERIN IN THE COLD

In work done with Dr. Poor on the virus of rabies, we compared the glycerin resistance of that virus with those of the diphtheria and tubercle bacilli. When acted on in the cold by glycerin, we found the diphtheria bacilli were alive after two weeks, while after thirteen months the tubercle bacilli were alive and virulent to guinea-pigs, thus showing a glycerin resistance as great for that organism as for the virus of rabies or vaccinia.

In our present work, we have continued our experiments on a larger series of organisms of a known nature, including the spirochete

of syphilis. The technic in these experiments was similar to that in the former work; vigorous cultures on solid media were covered with 3-5 c.c. of sterile, neutral glycerin and kept in the ice-box. At definite intervals, transfers of the culture were made to fresh media, solid and fluid, carrying over as little as possible of the glycerin. These transfers were then incubated.

TABLE 1
SHOWING RESISTANCE OF ORGANISMS TO GLYCERIN IN THE COLD

Organism	After 3 days*	After 6 days	After 14 days	After 21 days	After 30 days	After More Than One Month
<i>Proteus vulgaris</i>	+	+	—			
<i>B. prodigiosus</i>	—	—				
<i>Sta. pyogenes aureus</i>	+	++	+	±	One colony after 32 days
<i>Pneumococcus</i>	—	—				
<i>Streptococcus</i>	—	—	—			
<i>Meningococcus</i>	—	—	—			
<i>B. diphtheriae</i> 8.....	+	+	+	—	—	
<i>B. diphtheriae</i> 17.....	+	+	+	±	—	
<i>B. pyocyaneus</i>	—	—				
<i>B. mallei</i>	—	—				
<i>B. coli communis</i> (1)	++	++	+	+	±	Growing less vigorously — 5 colonies after 32 days ++ after 41 days
<i>B. coli communis</i> (2)	++	++	++	++	++	
<i>B. typhosus</i>	+	±	—			
<i>B. dysenteriae Shiga.</i>	—	—				
<i>V. cholerae</i>	±	—				
<i>B. tuberculosis</i> 72.....	+	—			
<i>B. tuberculosis</i> 305....	±	±	±	Very feeble growth after 50 days
<i>B. tuberculosis</i> 311....	—	—	—	
<i>B. tuberculosis</i> 422....	—	±	±	Feeble growth after 50 days
<i>B. tuberculosis</i> , Courmont	++	+	++	Vigorous after 50 days
<i>B. tuberculosis</i> , Dr. L.	±	±	Alive after 50 days
<i>B. tuberculosis</i> , Koeli	++	+	Alive after 50 days
<i>B. tuberculosis</i> , Ravenel	++	±	Very feeble growth after 50 days
<i>Spirochaeta pallida</i> ..	—	—				

* ++, vigorous growth; +, moderate growth; ±, feeble growth; —, no growth.

The glycerin resistance of the spirochete was tested as follows: A small piece of a nodule from an inoculated testis of a rabbit, which showed abundant spirochetes, was immersed in glycerin and kept in the ice-box. After seventy-two hours this piece was removed, emulsified, and inoculated into a rabbit's testis with negative results, while inoculations from the original material proved virulent. A culture of spirochetes from a strain received originally from Dr. Noguchi, was subjected to glycerin in the cold. Seventy-two hours later, transplants were made, which did not grow when incubated, while control transfers grew. These results were not surprising, since the

spirochaeta pallida is killed by drying. However, not all the organisms which resisted desiccation were glycerin resistant.

In our present work, the most resistant of the bacteria examined were the tubercle bacilli, transfers of which grew after the glycerin had been on the cultures for fifty days (they were not tested further), and then the staphylococcus and the colon bacillus, which were still alive after thirty to forty days. The typhoid bacillus did not resist more than six days. Table 1 shows the work in detail.

SUMMARY

Cultural experiments with the virus of vaccinia, with usual or special bacteriological methods, were negative. There was no evidence of a multiplication of the virus. The virus, streaked on agar plates and kept anaerobically, has remained alive for eight weeks at 33 C. An acid reaction of media appears favorable to the virus.

A comparative study of the action in the cold of glycerin on organisms of a known nature shows the resistance to be a variable one, not always paralleled by resistance to drying. Of the organisms tested, the tubercle bacilli were the most resistant, then the staphylococci and colon bacilli.

A STUDY OF THE SO-CALLED IMPLANTATION OF THE BACILLUS BULGARICUS *

ALFRED H. RAHE

(From the Department of Experimental Pathology, Cornell Medical College, New York City)

After Metchnikoff's announcement that certain lactic acid-forming bacilli found in the fermented milk Yalhourth were able to check the growth of putrefactive bacteria in the intestines, in all the articles published in attempted confirmation of his claims, the methods used to identify the bacillus are inadequately described. This work was undertaken with the purpose of determining, in the light of a better understanding of the cultural peculiarities of the bacillus bulgaricus, whether or not a true implantation took place.

In the human intestine there occur normally bacilli that culturally and morphologically resemble this bacillus very closely. The group of organisms, which includes the bacillus bulgaricus, is distinguished from other groups of bacteria by the ability of its members to grow in media containing considerable amounts of acid; because of this characteristic, these bacilli are called aciduric or acid-tolerant organisms. In a previous paper¹ the writer was able to show that, contrary to the usual statement, these bacteria grow luxuriantly in an ordinary laboratory medium, viz., *unneutralized* meat-peptone broth containing glucose or other suitable carbohydrate, and that agar prepared from this broth, with or without the addition of 0.2 percent of sodium oleate, is an excellent solid medium. It was also shown that while the bacillus bulgaricus, from its cultural and perhaps biochemical properties, also belongs to this group of sugar fermenting, acid-tolerating organisms, it differs from the bacteria of the type of the bacillus acidophilus in failing to ferment maltose. Some strains do not attack saccharose.

REVIEW OF THE LITERATURE

Bertrand and Duchacek² mention the failure of this organism to ferment maltose, but do not give the nature of the medium used. On the other hand

* Received for publication December 3, 1914.

1. Jour. Infect. Dis., 1914, 15, p. 141.

2. Ann. de l'Inst. Pasteur, 1909, 23, p. 402.

3. Rev. méd. de la Suisse romande, 1905, 25, p. 714.

4. Compt. rend Soc. de Biol., 1906, 60, p. 558.

Grigoroff³ and Cohendy,⁴ equally indefinite as to medium, claim an opposite result. This contradiction in evidence leads to the suspicion that faulty identification may have led, in at least some instances, to erroneous conclusions as to the implantation and survival of this bacillus in the intestine. Cohendy claims to have demonstrated that the organism becomes established in the intestine in eight days and survives for twelve days or less after feeding has been stopped. According to Belanowsky⁵ the bacillus becomes adapted to the human

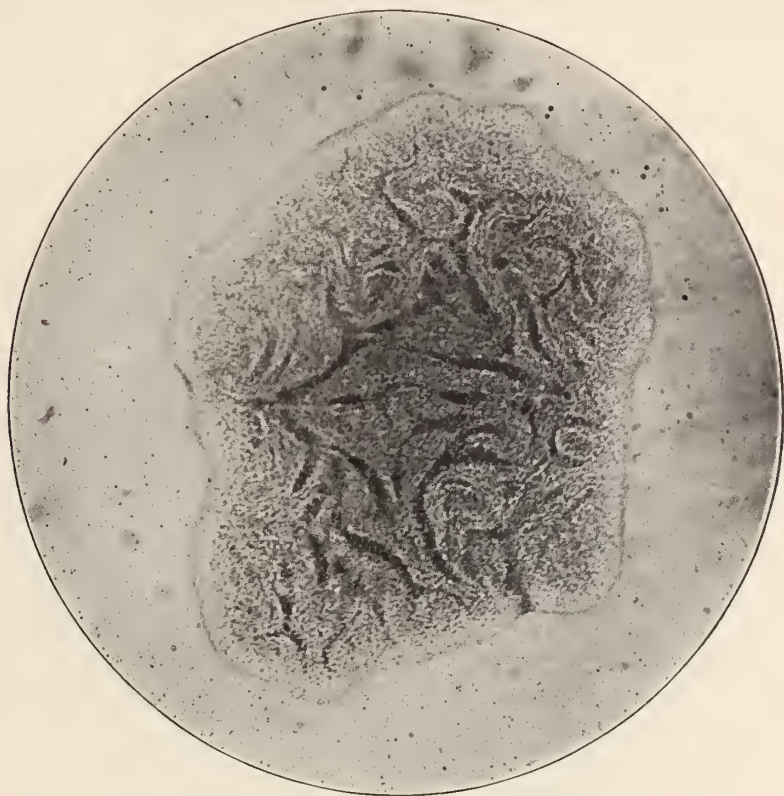


Fig. 1.—An atypical colony of the bacillus bulgaricus. No projections. $\times 60$.

intestine and survives for varying periods. Distaso and Schiller⁶ fed the organism to rats and concluded that it is impossible to force the growth of a foreign organism in the intestine. Herter and Kendall⁷ fed to monkeys Bacillac, a commercial fermented milk in which the bacillus did not occur in pure culture. The organism was recovered from the stools on the fifth day after feeding commenced. After fourteen days of exclusive feeding with this milk the organism was found in almost pure culture in the duodenum and jejunum, in

5. Ann. de l'Inst. Pasteur, 1907, 21, p. 991.

6. Compt. rend. Soc. de Biol., 1914, 66, p. 243.

7. Jour. Biol. Chem., 1908, 5, p. 293.

less numbers in the cecum, and hardly at all in the colon and rectum. In a personal communication to the writer, Dr. Kendall has described the method of isolation used in his investigation. A small amount of feces was seeded into milk and pure cultures obtained by repeated transfer in this medium. The organism was recognized by its complete parasitization to milk.

It seems certain that Herter and Kendall succeeded in isolating the organism ingested, but in the case of other workers the lack of



Fig. 2.—Colony of intestinal aciduric bacteria simulating that of the bacillus bulgaricus. $\times 90$.

cultural detail leaves one in doubt as to the correctness of their findings.

For the identification of the bacillus bulgaricus colony formation, morphology and quantitative estimation of acid production are insufficient. The colonies are usually described as resembling those of the bacillus anthracis in their loose texture, irregular outline, and forma-

tion of projections. While this so-called characteristic form is frequent, a more regular form, one that resembles the typical form only in its fissured surface, is also common (Fig. 1). During the course of this work there often occurred on the plates, made from the enrichment tubes which had been planted with feces, colonies of aciduric bacteria normal to the intestine that very closely resembled those of the bacillus bulgaricus (Figs. 2 and 3). During the period of ingestion



Fig. 3.—Colonies of the bacillus acidophilus with projections. About $\times 6$.

mixed colonies, composed of the bacillus bulgaricus and intestinal acid-tolerant bacilli, were common. In staining and shape the bacillus bulgaricus is identical with the bacillus acidophilus.

While some strains of the Bulgarian bacillus are capable of a high acid production, others do not produce so great an acidity as that formed by some strains of the bacillus acidophilus. The inability of the bacillus bulgaricus to utilize maltose constitutes its essential difference from the intestinal aciduric bacteria that coagulate milk.

In this investigation pure forty-eight-hour cultures of the *bacillus bulgaricus* were ingested in the quantities indicated on the charts. Stools were collected at regular daily intervals and treated as follows: Within a few minutes after the passage of the movement a representative sample of 0.5 gm. was accurately weighed and emulsified in 50 c.c. of normal salt solution. This suspension was shaken for three minutes and the grosser particles allowed to settle. A series of dilutions was prepared and 0.5 c.c. of each dilution was seeded into tubes containing 10 c.c. of milk having an acidity of plus 2.5 percent normal lactic acid. Milk, acidified or not, has been used as an enrichment medium by practically all of the workers in this field. After six days' incubation at 37 C. a loopful from each milk tube was streaked on the surface of hardened and dried meat-peptone-oleate-glucose agar. After forty-eight hours' incubation likely colonies were fished and maintained in the unneutralized glucose broth.

On the plates from the enrichment tubes there were, as might be expected, mixed colonies composed of intestinal aciduric organisms and the *bacillus bulgaricus*, but the latter could not be distinguished as such under the microscope. In most cases cultures from such mixed colonies gave an acidity in milk nearly as great as that of the pure cultures of the organism ingested. Growth in maltose broth showed the presence of intestinal organisms and plates made from the primary cultures in glucose broth invariably showed colonies of both organisms, while plates from the maltose broth showed colonies of bacilli only of the acidophilic type. Occasionally in the mixed cultures in milk the intestinal organism predominated and the acidity did not rise above plus 17.0 percent normal in six days.

An attempt was made not only to determine whether the organism ingested could survive in the intestine but also to determine to what extent it was present. To that end, in addition to the monkeys, human subjects presenting varying intestinal conditions were chosen. In all of the experiments that follow the culture was taken just before meals.

Subject A, female, 32 years old, in the third or fourth month of pregnancy. The stools were typically putrefactive. There was only a slight constipation. The average daily diet of Subjects A and C was as follows: fruit 407 gm., bread 147 gm., cereal 158 gm., meat 230 gm., vegetable 280 gm., cane sugar 27 gm., milk 100 c.c., dessert (bread pudding, tapioca, blanc-mange) 142 gm.

Chart 1 shows that the organism appeared in the stools of Subject A for the first time on the eighth day. For the period during which broth culture was fed the curve reaches its greatest height on the tenth day and this point is not passed during the increased ingestion in the incompletely recorded period of broth culture feeding that follows. With the milk culture the excretion reaches its greatest height, but

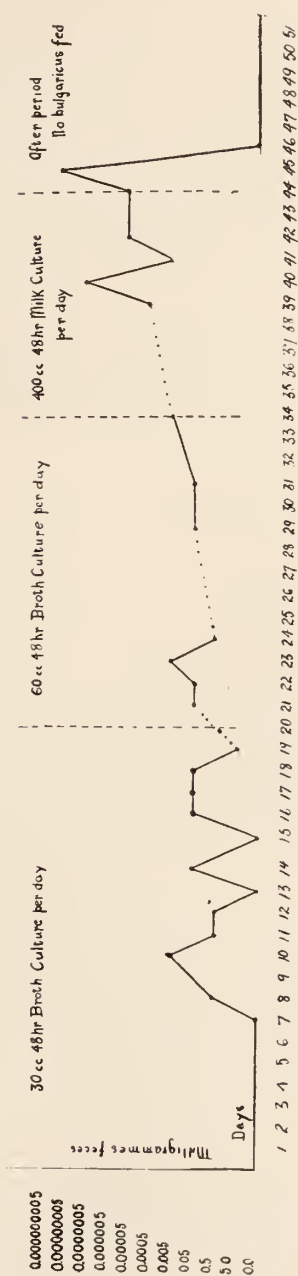


Chart 1 (Subject A).—Greatest excretion of the bacillus bulgaricus reached on day afterfeeding stopped.

the greatest elimination occurs on the day after feeding was stopped. The bacillus disappeared from the stools on the following day.

Subject B, male, aged 53. He subsisted both before and during the experiment entirely upon vegetable food. His stools were dry, almost odorless, and characterized by their high content of aciduric bacteria. One liter of a forty-eight-hour milk culture was taken per day. The bacillus bulgaricus appeared in the feces for the first time on the fifth day and the greatest excretion was reached on the sixth day. After its first appearance the organism was absent from two consecutive stools on two occasions. It maintained its level for one day after the feeding stopped and disappeared on the third day after the last ingestion.

The organism was fed to Subject B in enormous numbers. Altho diet and intestinal conditions were such as would be expected to favor its development, and the bacillus appeared in the stools three days



Chart 2 (Subject B).—Ingested organism absent on third day after feeding stopped.

earlier than in the preceding experiment, it survived but one day longer.

Subject C, male, 32 years old. The diet was of the same quantity and composition as that of Subject A. The feces were moderately putrefactive. The organism appeared on the second day following the first ingestion and survived for the same length of time as in the case of Subject B. As in the other two subjects, this bacillus disappeared completely from the stools within a few days after the ingestion of the culture was stopped and was not recovered again, altho the examinations were continued daily for a week. In one instance during the feeding of the culture the bacillus was absent from three consecutive stools.

Neither in this nor in the preceding experiments did the disappearance of the organism occur at times when several stools were passed during two days. Their absence must have been due to some other cause than a sudden elimination. Either the bacillus died off rapidly

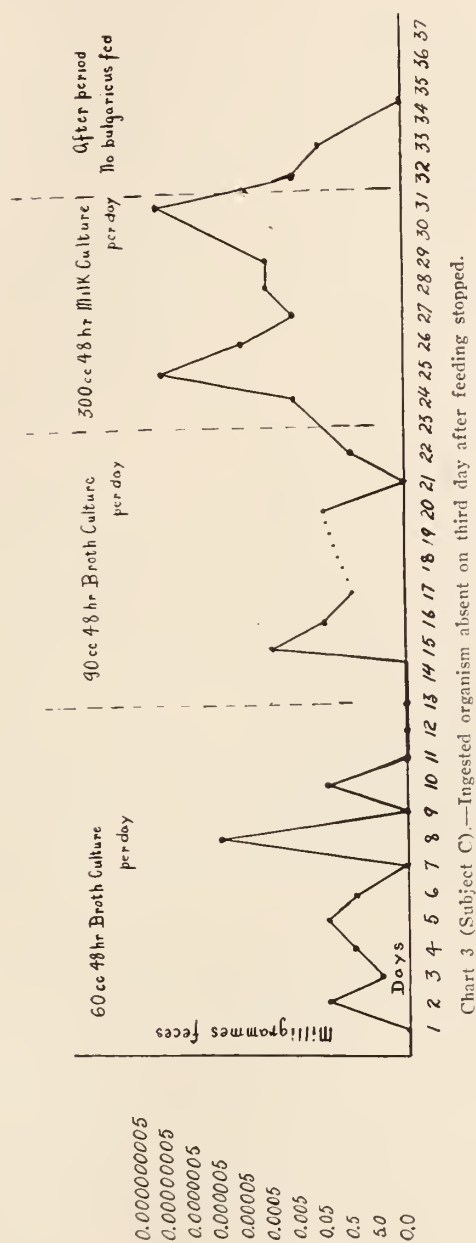


Chart 3 (Subject C).—Ingested organism absent on third day after feeding stopped.

or it was retained in the upper levels of the digestive tube and passed into the feces in numbers too small to be detected.

Subject W., female, 25 years old, had been taking the bacillus bulgaricus in tablets for several months. For eight weeks during which this subject received 60 c.c. of broth culture per day the stools were examined at irregular intervals. The organism was found present in nearly all of the stools tested and survived eight days after feeding stopped.

Altho in the above experiments the bacillus bulgaricus disappeared from the feces soon after ingestion stopped, it has not been proved that the organism does not survive for a longer period in the small intestine. It is possible that an organism might be able to adapt itself to the conditions prevailing in the duodenum and jejunum, but be unable to survive in the lower intestine, and so be absent from the stools.

In order to determine if such survival occurred, small Rhesus monkeys that had been fed the bacillus bulgaricus were killed at different intervals after the feeding was discontinued. Five-tenths of a gram of the contents of the different regions of the intestine were treated in the same way as the feces of the human subjects. The monkeys were fed twice a day and received the following average diet: one banana, 99 gm. boiled rice, one apple, 58 gm. bread, 62 gm. cabbage.

Monkey 1 was given fifty lactose tablets per day, each of which contained approximately 2,000 bacilli. The tablets were prepared as follows: after a heavy broth culture was centrifugalized, the deposited organisms were suspended in a very small amount of salt solution, and with lactose were formed into tablets. Fresh forty-eight-hour cultures were used and the tablets fed just before meals. After the thirteenth day the feeding was stopped and the animal killed on the day following. The bacillus bulgaricus was recovered in fairly large numbers from the jejunum, but not from the other parts of the digestive tube and feces.

Monkey 2 was fed 300 c.c. of forty-eight-hour milk culture per day for six days and cultured seven hours after the last feeding. The organism was found in large numbers in the duodenum, jejunum, ileum, and feces, and very scantily in the colon.

Monkey 3 received the same amount of culture for the same length of time as Monkey 2, but the feeding was stopped seven days before death. The bacillus was recovered in small numbers and only from the duodenum.

This series of experiments, while not complete, serves to show that the organism may survive in the small intestine after it has disappeared from the lower digestive tract and feces, tho it is probable that this survival is not permanent. Table 1 gives the results of these experiments.

Altho it is probable that the bacillus bulgaricus once flourished in the intestine of a warm blooded animal, as its preference for body temperature shows, the facts brought out in this investigation indicate that it is no longer capable of developing in the lower part of the digestive tube. Its absence from the feces, unless previously ingested in great numbers, is especially interesting in view of the fact that Hastings and Hammer⁸ have discovered an organism resembling the Bulgarian bacillus in milk, butter, and cheese. It is probable that their bacillus was an intestinal organism, but, since these authors do not state whether it attacks maltose, it is probable that they were dealing with an exceptionally active strain of the bacillus acidophilus.

TABLE 1
THE SURVIVAL OF THE *BACILLUS BULGARICUS* IN THE INTESTINES OF MONKEYS

Monkey	Fed	Period of Ingestion	Time After Last Ingestion	Duo-denum	Jejunum	Ileum	Colon	Feces
1.....	Fifty tablets per day.....	13 days	24 hrs.	—	+	—	—	—
2.....	300 c.c. 48-hr. milk culture..	6 days	7 hrs.	+	+	+	+	+
3.....	300 c.c. 48-hr. milk culture..	6 days	7 days	+	—	—	—	—
Control	Not fed.....	—	—	—	—	—

The sign + denotes the presence of the bacillus bulgaricus; the sign — denotes absence.

The mere recovery of an organism from the feces several days after the feeding has stopped does not prove that it has become adapted to the intestine, and, while the evidence developed in this investigation does not exclude the possibility of a slight multiplication of the bacillus bulgaricus in the lower part of the human digestive tract, it is obvious that nothing resembling a true implantation took place.

The charts show that the point of greatest excretion of the ingested organism is the same for all three subjects and represents an elimination of at least twenty million organisms per milligram feces. The average daily excretion for Subjects A, B, and C was about one million organisms per milligram.

Torrey,⁹ in an investigation of the fecal flora of typhoid patients on a high calory diet, found that the excretion of bacteria of the type of the bacillus acidophilus frequently reached one million organisms per milligram feces and in one instance was six times that amount.

8. Univ. of Wis. Agric. Exper. Sta. Research Bull. No. 6, 1909, p. 195.

9. Jour. Infect. Dis., 1915, 16, p. 72.

In the present investigation intestinal aciduric organisms were frequently encountered at very high dilutions of the feces and it is probable that their excretion paralleled if it did not exceed that of the bacillus bulgaricus, notwithstanding the enormous ingestion of the latter.

CONCLUSIONS

The bacillus bulgaricus is an organism readily distinguished from the intestinal aciduric bacteria.

The evidence indicates that this bacillus cannot become adapted to the human lower intestine.

The experiments with monkeys show that the bacillus bulgaricus is capable of an apparently limited survival in the upper intestine of these animals.

THE VARIABILITY OF TWO STRAINS OF STREPTOCOCCUS LACTICUS *

P. G. HEINEMANN

(From the Bacteriological Laboratory, University of Chicago, Chicago)†

Much attention has been given during recent years to methods of classification of streptococci. The literature has been fully discussed by several authors and a review here is unnecessary. Practically all the work reported was carried on by collecting streptococci from different sources and testing their fermentative powers on certain substances by the amounts of acid formed. Hemolysis, reaction in litmus milk, and morphology have also been taken into consideration. Rogers,¹ among others, has pointed out that morphology is of little constancy and depends largely on the kind of available food. Acid formation and hemolytic power have been considered by some to be of sufficient constancy to determine the origin of the culture with some degree of certainty. Whether or not these properties remain constant under changed conditions has not to my knowledge been systematically investigated.

In the work reported in this paper I have approached the subject from a novel viewpoint. Instead of isolating a large number of streptococci, I have selected two strains of the streptococcus lacticus and subjected them to definite conditions. The different tests were repeated at certain intervals during the progress of the work, the object of which was to determine in what manner reactions may be influenced by environment.

The following test substances were used to determine the amount of acid formed: dextrose, lactose, saccharose, raffinose, inulin, salicin, and mannite. One percent of these substances was dissolved in a medium prepared by covering chopped beef with water in the proportion of one liter of water to one pound of beef. After soaking overnight a culture of the bacillus coli was added and the mixture incubated at 37 C. for twenty-four hours. The medium was then heated to 60 C. and held at this temperature for two hours. Then, the temperature was raised to the boiling point and held there for thirty minutes. The meat was pressed out and the remaining meat mixed again with water and pressed a second time. The fluids were mixed

* Received for publication December 30, 1914.

†This work was aided by a grant from the American Dairy Research Association.

1. Jour. Agr. Research, 1914, 1, p. 491.

and the measure brought to the requisite amount, i. e. 1,000 c.c. fluid for each pound of meat. Ten liters were prepared at a time. In the boiling liquid 1 percent Witte peptone and 0.5 percent sodium chlorid were dissolved. After solution, 6 gm. calcium carbonate for each liter were added and the mixture was boiled until the reaction was about 0.3 percent acid to phenolphthalein. This method of neutralization was preferred to the usual one of adding sodium hydrate, since minute amounts of alkali render carbohydrates subject to easy decomposition, while they are relatively stable in an acid reaction. By using calcium carbonate, even the small amount of alkali necessary to produce a pink coloration with phenolphthalein is avoided. It required boiling with calcium carbonate from thirty minutes to an hour to obtain the desired reaction. The medium was then sterilized in large Erlenmeyer flasks without filtration, so that any acid that might form during sterilization would be neutralized by the excess of calcium carbonate. The medium was filtered in a boiling hot condition before the test substances were dissolved. Carbon dioxid was therefore completely removed and no calcium carbonate held in solution. Possible error from decomposed carbohydrates by the presence of alkali was thus avoided.

After solution of the test substances, the medium was distributed in small Erlenmeyer flasks and Nessler tubes. The object was to vary progressively the amount of available free oxygen to the amount of fluid. The largest amount of free oxygen was available in small Erlenmeyer flasks by the large surface offered by 20 c.c. of the fluid. Then, a series of Nessler tubes containing 20, 30, 40, and 50 c.c., respectively, were filled. Finally, another Nessler tube was filled with 50 c.c. and covered with boiled paraffin oil. By this method, each test substance was contained in six lots, each lot offering less free oxygen in proportion until finally anaerobic conditions had been established.

The chief object of the study was to determine whether the original fermentative ability, as indicated by amount of acid formed, would remain the same or be changed by animal passage of the strain. As the streptococcus lacticus is non-pathogenic in its natural condition, large doses are required to produce reactions in animals. The surface of a twenty-four-hour culture on North medium was suspended in physiological salt solution and injected subcutaneously into a rabbit, and the surface of another tube similarly prepared injected into a guinea-pig. The organism was recovered as soon as pronounced lesions appeared, usually in from two to three days. After twenty-four hours' cultivation on North medium, the recovered organism was again inoculated into a rabbit and a guinea-pig. This process was repeated a number of times with the result that virulence was greatly enhanced. Great caution was used when removing material from the lesions for recovery of the organism. The abdomen was shaved, washed in 0.2 percent mercuric chlorid solution and this washed off with alcohol. This procedure was followed before all injections. The material was removed with a platinum loop and streaked on North medium. Streptococci are widely distributed and unless care is exercised there is the possibility that some other strain is obtained instead of the desired one. That this did not happen is proved by cultural tests and chiefly by the fact that the changes in fermentation reactions were distinctly progressive. Suspicious cultures were discarded and another organism was isolated from the same lesion.

The original strains and all strains recovered from animals were cultivated on North medium and transferred after twenty-four-hours' growth to the Erlenmeyer flasks and Nessler tubes prepared in the described manner. Suitable wooden stands were used to hold a series of Nessler tubes. Thus, a strain of the streptococcus lacticus was inoculated into an Erlenmeyer flask containing

dextrose dissolved in the medium and into five Nessler tubes containing 20, 30, 40, 50, and 50 c.c., respectively; the last tube containing 50 c.c. was covered with oil after inoculation. The same scheme was carried out with flasks and tubes containing lactose, saccharose, raffinose, inulin, salicin, and mannite. In addition, blank tubes containing each of the test substances dissolved in the medium were prepared. All flasks and tubes were incubated at 37 C. After the lapse of three days the flasks and tubes were shaken and five c.c. removed with sterile pipettes. The 5 c.c. were mixed with 45 c.c. distilled water and, after addition of 1 c.c. of a 1 percent solution of phenolphthalein, titrated with 0.05 normal potassium hydrate solution. The acidity of the blank was deducted from the amount obtained in the inoculated tubes.

The organism was also streaked periodically on blood agar. Human blood and goat's blood were tested. Beef infusion agar was put into tubes, each one containing 9.5 c.c. agar. After melting and cooling to 55 C., 5 c.c. of the defibrinated blood were mixed with the agar, the mixture poured on Petri dishes, and incubated for twenty-four hours to eliminate contamination. A suspension of the organism was streaked on the surface of the blood agar with a platinum needle, the end of which was triangular and hammered flat.

Cultures were made from day to day on North medium in litmus milk and in dilute horse serum. This latter was prepared by diluting horse serum with five times its volume of water and sterilizing. After sterilization there was some precipitate in the diluted serum, but it remained liquid, which was the object of dilution. The milk cultures were observed after twenty-four hours, two days, three days, and ten days. Stains from all cultures were made after twenty-four hours. The milk cultures were incubated in duplicate, one series at 37 C. and the other at room temperature.

The routine outlined was carried on with the original cultures and repeated after each animal passage. At the conclusion of the work, the second strain, which had been passed through milk fifty-eight times on consecutive days, was again tested. In addition, a hemolytic strain, obtained through the kindness of Dr. E. C. Rosenow, was carried through some of the tests for comparison. Work with the first strain was completed before work with the second strain was commenced. The first series was not so complete as the second series, since additional tests were designed for the second series. However, the results of the two series are similar, so that one may be considered as confirmation of the results of the other.

The work will be taken up in the following order: isolation of strains of the streptococcus lacticus; morphology and ocular observations in media; virulence, and lesions produced; acid production in the seven test substances; and influence of amount of available oxygen.

ISOLATION OF STRAINS OF STREPTOCOCCUS LACTICUS

Strain 1 was isolated from ice cream. The liquefied cream was plated in several dilutions in dextrose litmus agar and after two days' incubation at 37 C. nine streptococcus-like colonies were transferred to slanted dextrose agar tubes. After twenty-four hours some of the surface growth was plated again. After colonies from the second set of plates were transferred to dextrose agar, stains were

prepared. The final selection gave a strain with the following characteristics: Rather small; frequent chains of six to nine members; many diplococci and decidedly round individual cocci. The Gram stain was positive and no capsules could be demonstrated by using Rosenow's capsule stain. Gelatin was not liquefied. Surface growths on agar and

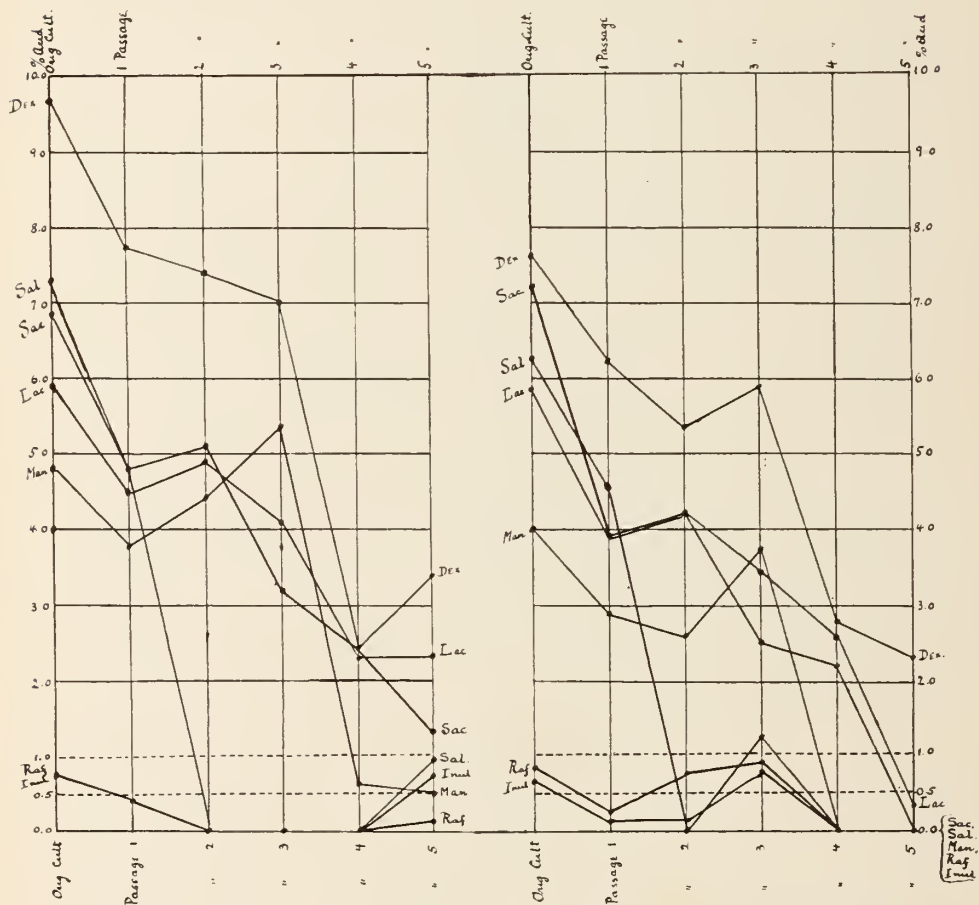


Chart 1.—Results of fermentations of strains obtained by animal passage of Strain 1, expressed in terms of normal acid.

on North media were composed of small colonies giving the appearance of a thin veil. Growth on North medium was always more abundant than on dextrose agar. Litmus milk was acidified after twenty-four hours and completely coagulated after forty-eight hours. The

coagulum was decolorized excepting a small pink ring at the surface. A flask containing 500 c.c. milk, sterilized in the autoclave, was inoculated with the contents of a tube of litmus milk containing twenty-four hours' growth. The milk in the flask was coagulated after twenty-four hours. The coagulum was smooth with very little whey. The taste was typical of lactic streptococci, excepting the pronounced cooked taste due to sterilization. This strain did not hemolyze either human or goat's blood agar. The culture has now been in the laboratory collection for nearly a year, during which time it was kept active by periodic transfers. At present it forms chains of enormous length, consisting occasionally of sixty to eighty members.

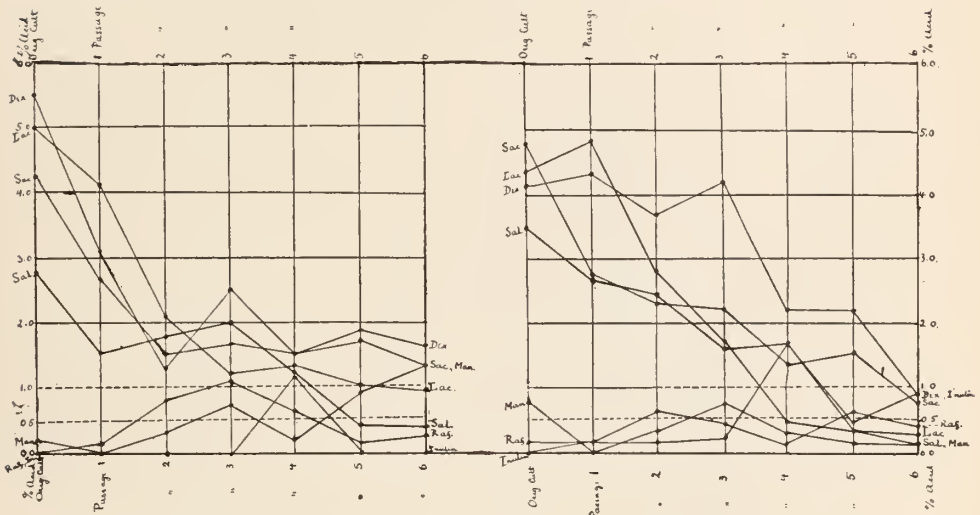


Chart 2.—Results of fermentations of strains obtained by animal passage of Strain 2, expressed in terms of normal acid.

Strain 2 was isolated from milk. Certified milk was incubated at 37 C. for two days. Plates were then prepared from the loppered milk in the same manner as previously described from ice cream. Eleven colonies were transferred to slanted dextrose agar. Of these, six were replated and studied carefully as they did not show distinct morphological differences. They were stained, inoculated on North medium, litmus milk, and gelatin. Surface cultures from North medium were suspended in physiological salt solution and inoculated into a rabbit and a guinea-pig. Streaks were made on human and goat's blood agar. It seemed important to make these tests to avoid

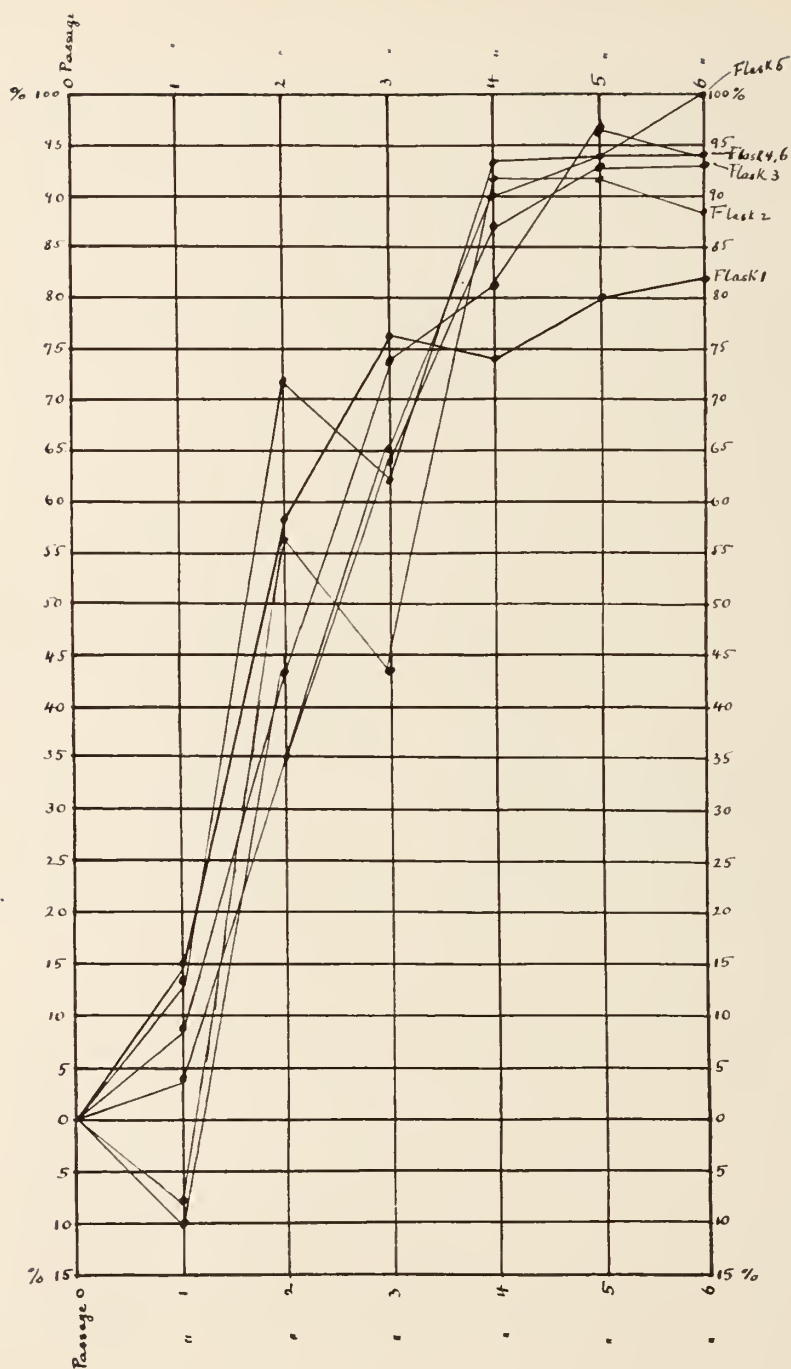


Chart 3.—Percent loss of fermentative power of Strain 2 in lactose. Rabbit passage.

selecting a strain that might be possessed of properties usually taken to indicate pathogenicity. The animals developed no severe lesions. A slight swelling and reddening at the point of injection were all that could be observed. There was no trace of green color or hemolysis on either human or goat's blood agar. One of the strains tested was finally selected. It resembled Strain 1 in all cultural characteristics, excepting that chain formation was less pronounced. Diplococcus forms were prominent and the diplococcus arrangement persisted in the short chains which occasionally were present. It did not liquefy gelatin, was gram-positive, and coagulated milk in forty-eight hours, both at room temperature and at 37 C. The growth on North medium was similar to that of Strain 1. When inoculated into 500 c.c. sterilized milk, the coagulum and taste were similar to those of Strain 1.

The hemolytic strain was passed through the same tests. It acted promptly on animals, causing pus formation; one rabbit died after twenty days and one guinea-pig recovered. Milk was not coagulated in ten days, but acid was produced. Morphologically, it did not differ from the strains of the *streptococcus lacticus*. The cocci were small and spherical, the chains contained up to ten members, the Gram stain was positive, and no capsules could be demonstrated. When inoculated into 500 c.c. sterilized milk, no coagulum was formed for ten days. No observation was made after this period. Gelatin was not liquefied and surface growth was delicate and veil like. Hemolysis was distinct on human and goat's blood agar.

MORPHOLOGY AND REACTIONS IN CULTURE MEDIA

Strain 1 was passed through litmus milk on thirty-six consecutive days. The morphology did not change, but the time for complete coagulation was reduced to eighteen hours. After the third rabbit passage and the fourth guinea-pig passage, milk was not coagulated in seventy-two hours. The culture was passed through horse serum on six successive days. Tendency to chain formation was more distinct in this medium than in milk. Chains of eight to ten members were more frequent. After the fourth rabbit passage diplococci were scarce, the culture consisting chiefly of chains, altho long chains were not observed. At no time was it possible to demonstrate capsules.

Observations on hemolysis were always made after twenty-four hours' incubation at 37 C. No hemolysis was observed after thirty-

six transfers through milk. Horse serum cultures were non-hemolytic. Animal passage gradually developed this property. After the third guinea-pig passage there was slight hemolysis. It was distinct in the thinner parts of the plate but not clear in the thicker parts. After the fourth guinea-pig passage, hemolysis was distinct in all parts of the plate. The clear ring was small. Hemolysis was distinct after the third rabbit passage. Two of the plates were contaminated with a few colonies of the bacillus subtilis. Whenever this occurred the colonies near the bacillus subtilis colonies had a greenish tinge. This phenomenon has been reported by Rosenow.²

More extensive studies were made with Strain 2. The original culture coagulated milk completely in somewhat less than forty-eight hours. After thirteen transfers through milk, coagulation was complete in twenty-four hours at 37 C. and after eighteen hours at room temperature. Chains appeared in all milk cultures, but diplococci were most numerous. The chains were chiefly composed of diplococci, and were frequently seen with eight to ten members. Chain formation in the room-temperature cultures seemed to be more common than at 37 C. After thirty-three transfers through milk, chains of twelve to fourteen pairs were frequently observed. Capsules were not demonstrated at any time.

A culture obtained through the kindness of Professor E. G. Hastings was compared with Strain 2. This culture was found in almost pure culture in the milk from a cow without udder trouble. The cow was kept under observation by Professor Hastings, but no udder lesions developed subsequently. This streptococcus coagulated milk in twenty-four hours and agreed morphologically in every respect with Strain 2. The length of chains also was about the same. This organism was non-pathogenic to rabbits and guinea-pigs. No attempt was made to produce virulence of this strain by animal passage.

Strain 2 and the hemolytic strain were passed through horse serum ten times. Chains of considerable length and composed of diplococci were predominant after two transfers.

Milk cultures from the second rabbit passage did not coagulate milk in seventy-two hours either at 37 C. or at room temperature. After animal passage, strains frequently showed numerous diplococci of peculiar shape. The individual cocci seemed to be elongated at right angles to the chain axis, thus resembling the so-called picket-

fence ("stakett") form. Guinea-pig passage did not seem to affect the power of coagulating milk as much as rabbit passage. Even after the sixth guinea-pig passage, milk was completely coagulated after forty-eight hours. Decolorization of the litmus, however, was not complete as it was in earlier cultures.

The above-noted picket-fence diplococci became more numerous with the number of passages. Cultures from the third rabbit passage occasionally showed several of these diplococci joined to a chain.

Cultures from the second rabbit passage hemolized both human and goat's blood agar. The clear circles of hemolysis were small. The hemolytic power persisted through cultures from all subsequent passages. Cultures from guinea-pig passages showed no hemolysis before the fifth passage.

VIRULENCE AND LESIONS PRODUCED

Guinea-pig Passages.—The first injection of Strain 1 produced a small, indurated area. On opening after three days, a small amount of blood and serum was discharged which gave a pure culture of streptococci. The second injection caused necrosis of tissue and the formation of a small pocket of pus. A smear from the pus showed streptococci in pure culture. The amount of pus increased with succeeding passages, but no generalized infection took place, and the animals eventually recovered.

A guinea-pig was injected with 5 c.c. of a seventh transfer of Strain 1 in horse serum. The animal died after eleven days. The peritoneal and pleural cavities were filled with a bloody fluid; the lungs were normal; the liver filled with small pus centers; and the spleen was very dark. Streptococci were recovered from the heart's blood, the peritoneal fluid, and the spleen.

The first injection of Strain 2 produced a small, indurated area, somewhat swollen and reddened. The organism was recovered after three days and injected into Guinea-pig 2. A small pus pocket formed. In the third guinea-pig passage, the indurated area was large with considerable pus. The symptoms increased until in the fifth animal an area covering a large part of the abdomen was indurated and swollen. A large pus pocket had formed and the animal died after twenty-two days. No autopsy was made as post mortem infection had taken place before the death of the animal was discovered. The sixth guinea-pig died after nineteen days. There was much pus over the

abdomen, and the inguinal glands were enlarged. Streptococci were recovered from the pus and the heart's blood. The seventh guinea-pig died after twelve days. No lesions were found in internal organs. On the abdomen a hard lump was found and much pus of tough consistency. Streptococci were recovered from the pus, the heart's blood, and the peritoneal fluid. The first four guinea-pigs recovered.

A culture of the original strain was injected into the peritoneal cavity of a guinea-pig without any apparent effect.

Rabbit Passages.—Virulence was acquired in shorter time and in greater measure by rabbit than by guinea-pig passage. The first injection of Strain 1 produced a soft, reddish area. On opening a serous fluid was discharged, which gave a pure culture of streptococci. The second injection produced a much larger area with a small center of pus. The third injection produced a large, inflamed area filled with pus which contained streptococci in pure culture. The rabbit had considerable temperature. The fourth injection produced similar, but more severe, symptoms. This rabbit died after fifteen days. The inguinal glands were swollen and the peritoneal and pleural cavities contained pus. Streptococci were recovered from the pus, the heart's blood, the liver, and the kidney. The fifth injection produced a large amount of pus and the animal became emaciated, but recovered.

The first injection of Strain 2 produced a small hyperemic area. There was a small amount of serum, which contained streptococci in pure culture. The second injection produced a small amount of pus. The third and fourth injections produced inflamed areas and pus in larger quantities. The fifth injection was followed by the formation of an enormous swelling containing some pus and a large amount of serum. This rabbit died after twenty-one days. The inguinal glands were enlarged, the kidneys congested, and pus foci were found in the lungs. Streptococci were recovered from the heart's blood and the pus in the lungs. After the sixth injection a large swelling developed which was filled with pus. The rabbit died in fifteen days. The inguinal glands were extraordinarily large. The other lesions were similar to the fifth rabbit. Streptococci were recovered from the pus, lungs, heart's blood, kidneys, and inguinal glands.

The hemolytic strain was injected subcutaneously into a rabbit. The animal died in twelve days. The inguinal glands were swollen and inflamed, pus was found at the point of injection, in the lungs, and peritoneum, and the kidneys, liver and spleen were congested. The organism was recovered from all organs and the heart's blood.

TABLE 1
AMOUNT OF ACID PRODUCED BY STREPTOCOCCUS LACTICUS (STRAIN 1)

Substance	Flask	Original Culture	Rabbit Passage					Original Culture	Guinea-pig Passage			
			1	2	3	4	5		1	2	3	4
Dextrose.....	1	9.7	7.8	7.4	7.0	2.4	3.2	9.7	6.8	6.8	6.2	2.6
	2	8.1	6.1	6.1	6.1	2.0	2.3	8.1	6.1	6.4	6.5	3.7
	3	7.3	6.9	5.9	5.9	2.3	2.1	7.3	6.1	6.3	6.8	2.1
	4	7.5	6.4	5.9	5.9	2.6	2.5	7.5	6.2	6.3	6.5	2.0
	5	7.5	6.9	5.8	5.9	2.8	2.3	7.5	6.1	6.4	6.5	1.3
	6	7.6	6.2	5.3	5.9	2.8	2.3	7.6	5.9	6.0	6.6	2.5
Lactose.....	1	6.0	4.5	4.9	4.1	2.3	2.3	6.0	4.1	4.2	4.9	1.5
	2	6.1	4.1	4.7	3.4	2.6	0.0	6.1	3.7	3.8	3.9	0.3
	3	6.1	4.2	4.5	3.4	2.7	0.1	6.1	3.8	3.8	4.1	0.6
	4	5.9	4.0	4.3	3.2	2.9	0.2	5.9	3.9	3.9	4.1	0.0
	5	5.7	4.1	4.1	3.1	2.8	0.5	5.7	4.3	3.8	4.2	0.0
	6	5.9	3.9	4.2	3.4	2.6	0.3	5.9	3.3	3.9	4.1	0.9
Saccharose.....	1	6.9	4.8	5.1	3.2	2.4	1.3	6.9	5.5	5.1	5.5	1.1
	2	6.7	4.1	3.9	2.3	2.3	0.4	6.7	4.5	5.1	5.0	1.1
	3	6.9	4.0	4.9	2.3	2.2	0.1	6.9	4.4	4.7	5.2	0.0
	4	6.8	4.3	4.7	3.1	2.0	0.2	6.8	4.5	5.3	5.2	0.0
	5	7.1	4.1	4.6	1.9	2.1	0.3	7.1	4.5	5.3	5.4	0.0
	6	7.2	3.9	4.2	2.5	2.2	0.1	7.2	4.6	5.3	5.4	0.9
Raffinose.....	1	0.7	0.4	0.0	0.0	0.0	0.1	0.7	0.4	4.7	1.1	0.7
	2	0.9	0.3	0.2	0.5	0.9	0.0	0.9	0.2	2.5	0.0	0.0
	3	0.8	0.3	0.5	0.6	0.0	0.0	0.8	0.2	2.5	0.0	0.0
	4	0.9	0.3	0.6	0.5	0.0	0.0	0.9	0.2	3.7	0.0	0.0
	5	0.9	0.3	0.3	0.7	0.0	0.0	0.9	0.2	2.7	0.0	0.0
	6	0.8	0.2	0.7	0.9	0.0	0.0	0.8	0.3	7.7	0.0	0.0
Inulin.....	1	0.7	0.4	0.4	0.2	0.0	0.7	0.7	0.4	1.5	0.0	0.6
	2	0.6	0.1	0.0	2.0	0.0	0.0	0.6	0.3	2.4	0.0	0.0
	3	0.6	0.2	0.1	0.7	0.0	0.0	0.6	0.2	2.4	0.0	0.0
	4	0.7	0.2	0.1	0.8	0.0	0.0	0.7	0.2	2.9	0.0	0.0
	5	0.8	0.2	0.2	0.8	0.0	0.0	0.8	0.2	2.7	0.0	0.0
	6	0.6	0.1	0.1	0.8	0.0	0.0	0.6	0.2	3.1	0.3	0.0
Salicin.....	1	7.3	4.8	0.0	0.0	0.0	0.9	7.3	5.7	0.1	6.3	0.5
	2	6.1	4.7	0.0	1.1	0.0	0.0	6.1	4.7	0.7	6.0	0.3
	3	6.1	5.4	0.0	1.1	0.0	0.0	6.1	4.6	1.3	6.0	0.0
	4	5.9	4.7	0.0	1.1	0.0	0.0	5.9	4.8	0.6	5.8	0.0
	5	6.3	4.7	0.0	1.2	0.0	0.0	6.3	4.7	1.2	5.8	0.0
	6	6.3	4.5	0.0	1.2	0.0	0.0	6.3	4.6	1.5	5.5	0.0
Mannite.....	1	4.8	3.8	4.4	5.3	0.6	0.5	4.8	3.9	4.6	4.9	0.7
	2	3.7	3.7	2.9	2.7	0.0	0.0	3.7	2.8	3.6	3.4	0.0
	3	3.8	2.9	3.0	3.3	0.0	0.0	3.8	2.8	3.6	3.3	0.0
	4	3.6	3.6	2.7	2.6	0.0	0.0	3.6	2.5	2.7	3.4	0.0
	5	3.7	2.9	2.9	2.5	0.0	0.0	3.7	2.4	2.7	3.4	0.0
	6	4.0	2.9	2.6	3.7	0.0	0.0	4.0	2.9	3.2	2.8	0.0

TABLE 2
 AMOUNT OF ACID PRODUCED BY STREPTOCOCCUS LACTICUS (STRAIN 2)

Substance	Flask	Original Culture	Rabbit Passage						Original Culture Cul.	Guinea-pig Passage						
			1	2	3	4	5	6		1	2	3	4	5	6	7
Dextrose....	1	5.5	3.1	1.3	2.5	1.5	1.9	2.6	5.5	3.9	3.9	3.3	3.1	2.7	2.3	1.6
	2	5.2	4.0	3.6	3.0	3.2	1.9	1.4	5.2	5.4	4.9	4.6	3.1	3.0	2.8	1.6
	3	5.1	4.3	3.1	2.7	1.6	1.7	1.2	5.1	5.0	5.1	4.1	3.0	2.9	3.0	1.5
	4	5.0	4.1	3.0	1.5	1.1	1.4	1.0	5.0	4.9	5.1	3.8	3.1	2.2	3.0	1.3
	5	5.1	4.0	3.1	1.7	1.1	1.4	0.9	5.1	4.9	5.4	3.7	3.4	3.2	2.4	2.9
	6	4.1	4.3	3.7	4.2	2.2	2.2	0.9	4.1	4.4	4.7	4.0	1.1	3.2	2.9	2.2
Lactose....	1	5.0	4.1	2.1	1.2	1.3	1.0	0.9	5.0	5.2	5.1	3.8	2.5	2.9	1.8	0.9
	2	4.9	5.5	2.1	2.8	0.4	0.4	0.6	4.9	5.1	4.7	4.1	1.6	2.9	2.9	0.9
	3	4.6	4.4	3.0	1.7	0.6	0.3	0.3	4.6	5.1	4.7	4.1	3.1	3.1	1.4	0.9
	4	5.3	4.8	3.0	1.4	1.0	0.2	0.3	5.3	5.0	4.8	3.7	3.1	3.1	1.7	1.1
	5	5.1	4.3	1.5	2.0	0.4	0.3	0.0	5.1	5.1	4.8	4.4	3.1	2.9	1.6	0.1
	6	4.3	4.8	2.8	1.7	0.5	0.3	0.3	4.3	5.1	5.5	4.5	2.9	1.7	3.0	0.8
Saccharose.	1	4.2	2.7	1.5	1.7	1.3	1.7	1.3	4.2	2.5	2.7	2.8	2.4	2.2	2.9	2.2
	2	4.6	3.6	3.1	3.2	1.4	1.6	1.3	4.6	4.6	4.7	2.1	2.7	2.8	2.6	2.1
	3	4.7	3.2	3.2	1.8	1.4	1.5	1.4	4.7	4.5	4.9	2.1	2.9	1.0	1.9	2.0
	4	4.6	3.1	3.2	3.3	1.1	1.4	1.2	4.6	4.7	4.9	2.2	2.8	2.7	2.8	1.6
	5	4.8	3.5	3.3	2.8	1.1	1.4	1.3	4.8	4.8	4.9	3.0	3.1	2.9	1.8	1.6
	6	4.8	2.8	2.3	2.2	1.3	1.5	0.7	4.8	4.7	4.8	2.9	2.4	1.6	2.9	2.9
Raffinose...	1	0.0	0.1	0.8	1.1	0.6	0.1	0.2	0.0	0.0	0.0	0.5	1.8	1.1	0.7	0.0
	2	0.1	0.1	0.7	0.2	0.3	0.6	0.3	0.1	0.1	0.1	0.0	1.6	2.0	1.1	0.9
	3	0.1	0.0	0.6	0.3	0.3	0.1	0.3	0.1	0.0	0.0	0.0	0.9	1.2	0.9	0.2
	4	0.0	0.0	0.6	0.4	0.1	0.0	0.3	0.0	0.0	0.0	0.5	1.0	1.7	1.8	0.7
	5	0.0	0.1	0.8	0.3	0.2	0.1	0.2	0.0	0.0	0.6	0.0	1.7	1.8	0.9	0.3
	6	0.1	0.1	0.6	0.4	0.1	0.6	0.4	0.1	0.3	0.6	0.8	1.6	1.9	0.9	0.3
Inulin.....	1	0.0	0.1	0.0	0.0	1.2	0.0	0.0	0.0	0.5	0.9	0.1	1.2	1.1	0.0	0.9
	2	0.0	0.1	0.6	0.2	0.6	0.2	0.8	0.0	0.2	0.0	1.2	1.0	0.9	0.0	0.2
	3	0.0	0.0	0.6	0.3	0.7	0.3	1.0	0.0	0.1	0.0	1.2	1.1	0.8	0.3	0.3
	4	0.0	0.0	0.6	0.2	0.6	0.1	0.7	0.0	0.0	0.0	0.0	1.2	0.8	0.6	0.3
	5	0.0	0.2	0.1	0.2	0.6	0.2	0.9	0.0	0.1	0.0	0.1	1.2	0.8	0.1	0.2
	6	0.0	0.1	0.1	0.2	1.7	0.4	0.9	0.0	0.2	0.0	0.0	1.4	1.0	0.6	0.2
Salicin.....	1	2.8	1.5	1.8	2.0	1.2	0.4	0.3	2.8	2.4	2.9	2.4	2.5	1.6	1.6	1.9
	2	3.1	2.5	2.2	1.8	0.6	0.4	0.2	3.1	3.2	3.5	2.2	1.8	2.1	2.2	2.1
	3	3.7	2.6	2.0	1.7	0.7	0.2	0.3	3.7	3.4	3.2	2.2	2.2	1.8	1.9	2.1
	4	3.4	2.9	1.6	1.9	0.6	0.3	0.1	3.4	3.2	3.5	2.3	2.1	1.9	2.1	1.9
	5	3.7	2.6	1.5	1.9	0.6	0.2	0.1	3.7	3.1	3.2	2.3	2.4	2.1	2.3	2.1
	6	3.5	2.7	2.4	1.6	1.7	0.3	0.1	3.5	3.3	3.2	2.3	2.3	2.2	2.2	1.8
Mannite....	1	0.2	0.0	0.3	0.7	0.2	0.9	1.3	0.2	0.1	0.9	0.0	1.9	1.2	1.6	0.3
	2	0.1	0.0	0.4	0.2	0.6	0.2	0.3	0.1	0.2	0.8	0.0	1.8	1.1	1.1	0.1
	3	0.3	0.0	0.3	0.3	0.4	0.2	0.3	0.3	0.0	0.8	0.0	1.6	0.9	1.1	0.2
	4	0.1	0.0	0.2	0.2	0.3	0.2	0.2	0.1	0.0	0.2	0.0	1.3	1.0	1.1	0.0
	5	0.2	0.0	0.3	0.2	0.2	0.0	0.1	0.2	0.1	0.1	0.0	1.4	1.0	1.2	0.3
	6	0.7	0.0	0.3	0.7	0.3	0.1	0.1	0.7	0.1	0.0	0.3	1.2	0.9	1.0	0.3

FERMENTATION REACTIONS

Both strains were inoculated into broth containing 1 percent of the test substances. This was repeated after each animal passage through rabbits and guinea-pigs and a control test of Strain 2 made of the milk culture after fifty-eight transfers through litmus milk. This was done in accordance with the technic outlined. In Tables 1 and 2, which show the quantities of acid formed, the figures represent the number of cubic centimeters of normal alkali required to neutralize the solution after the amount of acid found in the blank has been deducted.

TABLE 3

AMOUNT OF ACID PRODUCED BY STREPTOCOCCUS LACTICUS (STRAIN 2) AFTER FIFTY-EIGHT TRANSFERS THROUGH LITMUS MILK COMPARED WITH THE AMOUNT OF ACID PRODUCED BY THE ORIGINAL STRAIN

Flask	Dextrose		Lactose		Saccharose		Raffinose		Inulin		Salicin		Mannite	
	Pas- sage	Orig- inal	Pas- sage	Orig- inal	Pas- sage	Orig- inal	Pas- sage	Orig- inal	Pas- sage	Orig- inal	Pas- sage	Orig- inal	Pas- sage	Orig- inal
1.....	3.5	5.5	3.3	5.0	3.2	4.2	0.1	0.0	0.1	0.0	2.6	2.8	0.3	0.2
2.....	4.9	5.2	4.8	4.9	3.2	4.6	0.1	0.1	0.1	0.0	3.4	3.1	0.0	0.1
3.....	4.9	5.1	4.9	4.6	4.6	4.7	0.1	0.1	0.1	0.0	3.2	3.7	0.2	0.3
4.....	4.8	5.0	4.7	5.3	4.0	4.6	0.1	0.0	0.1	0.0	3.2	3.4	0.1	0.1
5.....	4.9	5.1	4.9	5.1	4.2	4.8	0.1	0.0	0.1	0.0	3.4	3.7	0.2	0.2
6.....	4.9	1.1	4.9	4.3	4.3	4.8	0.1	0.1	0.1	0.0	3.3	3.5	0.2	0.7

In the preceding tables, the column "flask" refers to the different exposures to free oxygen. Flask 1 is the Erlenmeyer flask containing 20 c.c. of the medium and offering a large surface to the air; Flask 2 is the Nessler tube with 20 c.c.; Flask 3, the Nessler tube with 30 c.c.; Flask 4, the Nessler tube with 40 c.c.; Flask 5 the Nessler tube with 50 c.c.; and Flask 6, the Nessler tube with 50 c.c. covered with oil for anaerobic cultivation.

For convenience, the results of Flasks 1 and 6 of each series are plotted in Charts 1 and 2. The figures at the top represent the number of the passage and the figures on the side the number of cubic centimeters of normal acid required for neutralization. Dotted lines appear at 1 percent and at 0.5 percent. Winslow³ takes 0.5 percent as the dividing line between acid formers and non-acid formers. Rogers⁴ takes 1 percent as the dividing line, while Hopkins and Lang⁵

3. Jour. Infect. Dis., 1910, 7, p. 1.

4. Ibid.

5. Ibid., 1914, 15, p. 63.

take 0.8 percent. Whichever amount is taken as indicative of acid production has bearing on the interpretation of the result, so that under one scheme a certain substance may be considered as having been fermented, while under another scheme the same substance would be considered as not having been fermented.

I have also figured the percentage of loss of fermentative ability by taking the amount of acid formed originally in the test solution as 0 and then plotting the percentages of loss in ascending curves. Two sets of charts were prepared according to these figures, one set being arranged according to flasks and the other according to the test-substance. Of the thirty charts prepared, Chart 3 is representative, showing the loss of lactose.

DISCUSSION OF RESULTS

A study of morphology leads to the assumption of three facts. Chain formation is favored by cultivation in media containing blood-serum in the absence of carbohydrates. Chain formation is favored also by animal passage. It is not clear from these studies what conditions are required for the production of picket-fence-shaped streptococci. Whether this is due to some particular substance in the medium or whether only certain strains are able to assume this form is not indicated. Perhaps both conditions are essential. Hemolytic power may be developed by animal passage. The ability to coagulate milk increases with frequent transplants through milk and decreases with increasing virulence. As will be shown later, coincident with increasing virulence is decrease of ability to produce acid from carbohydrates. It may be assumed therefore that the decreasing ability to ferment lactose reaches a stage when not sufficient acid is produced to coagulate the milk. The ability to coagulate milk decreased more rapidly by rabbit passage than by guinea-pig passage, while virulence increased more rapidly by rabbit passage than by guinea-pig passage.

The increase of virulence by animal passage has been discussed by me in an earlier paper.⁶ The results obtained in this work are in agreement with previously reported work and need not be discussed again.

The fermentation reactions are of particular interest. Several papers have been published recently dealing with the variability in fermentation reactions of several species of bacteria. Goodman⁷ suc-

6. *Ibid.*, 1907, 4, p. 87.

7. *Ibid.*, 1908, 5, p. 421.

ceeded in producing a high acid and a low acid form of diphtheria bacilli from one strain. Winslow and Walker⁸ found acid formation by the bacillus paratyphosus relatively stable. These authors made no attempt at variation of environment. Buchanan and Truax⁹ tested inheritance of acid production by the streptococcus lacticus. They conclude that "impressed variations do not appear to be inheritable." Rettger and Sherrick¹⁰ conclude from a study of several organisms that "bacterial variation, at least of the fluctuating type, may be brought about by what may be termed artificial selection." These authors suggest that by animal passage avirulent individuals may be eliminated and that by repeated passage elimination takes place repeatedly with production of a pure pathogenic type.

The changed fermentation reactions resulting from animal passage of the streptococcus lacticus in my experiments may not be constant. Possibly by recultivating the strains, the fermentative power of which was reduced, the original acid production might be re-established. Rettger's theory of selection by animal passage would receive support if the acquired ability should prove to remain constant. This problem awaits further experimentation.

Study of Chart 1, which gives the results of fermentations of strains obtained by animal passage of Strain 1, shows that the fermentative ability of the organism on the test substances is gradually reduced. Salicin is not fermented after the third passage through rabbits and not after the fourth passage through guinea-pigs. After the fifth passage, dextrose, lactose, and saccharose are still fermented after passage through either animal, but the quantity of acid produced is reduced. Mannite is not fermented after the fourth passage. If 0.5 percent acid is taken as dividing line we find that the small amount of acid formed from raffinose and inulin is lost after the first passage, but is regained after the fifth passage. In Flask 2, after the fifth rabbit passage, dextrose only is fermented, while after the fourth guinea-pig passage dextrose and saccharose are fermented. The amount of acid produced from saccharose is so small, however, that probably if a fifth passage had been made it would have been lost entirely.

It does not seem profitable to mention singly the results obtained in all the flasks by both strains. In a general way it may be stated

8. *Ibid.*, 1909, 6, p. 90.

9. *Ibid.*, 1910, 7, p. 680.

10. *Jour. Med. Research*, 1911, 24, p. 265.

that fermentative ability is gradually reduced by animal passage and that in due time some of the substances yield less than 0.5 percent acid and must be considered non-fermentable. In tubes with reduced oxygen supply, the decrease is more rapid than with free access of oxygen. Thus, the smallest loss is found in Flasks 1 and 2, while the following flasks show a relatively larger percentage of loss. However, the number of the experiments was not large enough to assign a definite ratio for the flasks. The relative decrease of amount of acid formed by rabbit passage is smallest in dextrose, next in saccharose, then follow lactose and salicin. By guinea-pig passage the relative loss in acid formation is smallest in dextrose, followed by saccharose, salicin, and lactose in the order named.

Whether the loss of acid production is due to actual loss of fermentative ability or to reduced growth may be questioned. This possibility was borne in mind during the progress of the work. No actual counts of bacteria were made from each flask, but the turbidity, which appeared after shaking, seemed uniformly similar to the naked eye. In this connection, it was noted that as the chains became more pronounced after animal passages the sediment became heavier and the supernatant fluid clearer.

A few words may be said in reference to the more complex substances, raffinose, salicin, and mannite. After the second rabbit passage salicin was not fermented; after the third, a small amount of acid was formed; and after the fourth and fifth passages no acid was formed. This holds good for Strain 1. For Strain 2 the curves are similar, but the actual differences much smaller. Raffinose and inulin have interesting curves. After the third rabbit passage of Strain 1 and the second guinea-pig passage, both these substances are fermented, but they are not fermented after the fourth rabbit passage and the third guinea-pig passage. I have no explanation for why these more complex substances should be fermented with moderate oxygen supply, but not with free access or total exclusion of oxygen. Mannite was fermented by the original culture of Strain 1, but not by Strain 2. In the fourth and fifth guinea-pig passages, however, there are small amounts of acid formed by Strain 2.

It may be stated in a general way that the acid-producing power of the two strains of the streptococcus lacticus is reduced as the number of animal passages increases. This decrease is rapidly pushed to a point where some of the substances, notably salicin and mannite,

are practically not fermented. As a rule, dextrose and saccharose are fermented more persistently than lactose. The figures suggest that more complex substances (raffinose and inulin) may be fermented after animal passage when the original did not ferment them. These two substances are fermented chiefly when the supply of free oxygen is reduced, but not eliminated. The reason is not clear and extensive experiments will have to be made to determine the exact nature of this phenomenon.

If we do not consider smaller amounts of acid than 1 percent, Strain 1 of the *streptococcus lacticus* before and after animal passages may be classified as fermenting dextrose, lactose, saccharose, salicin, and mannite; or dextrose, lactose, saccharose, and mannite; or dextrose, lactose, and saccharose; or dextrose and saccharose; or finally dextrose only. On the other hand, it may be classified as fermenting also raffinose and inulin.

Strain 2 may be classified as fermenting dextrose, lactose, saccharose, and salicin; or dextrose, lactose, saccharose, raffinose, and salicin; or dextrose, lactose, saccharose, and mannite; or dextrose, lactose, and inulin; or saccharose only; or finally as fermenting none of these seven substances. This consideration emphasizes that the ability to produce acid from test substances can be indicative only of the immediate source of the strain, but cannot serve as a permanent method of classifying streptococci into classes, species, or varieties.

Fermentation results under conditions of the experiments are changed materially and progressively. If we are able to determine the immediate source of a strain of *streptococcus* by testing its power to produce acid from certain test substances, it becomes necessary to find, if possible, whether conditions have prevailed which may have increased or decreased fermentative ability. In a general way, it may be assumed that streptococci that have been in contact with animal lesions, no matter whether these lesions were caused by the streptococci themselves or by other means, have low fermentative ability. Streptococci that have lived on food rich in carbohydrate show high fermentative ability. We can readily understand how an accidental injury to the udder of a cow, for instance, may favor multiplication of the *streptococcus lacticus*, which is ordinarily found in milk, and finally production of a variety which is pathogenic, of low fermentative ability, and hemolytic. We may assume that milk usually contains streptococci of different properties and that some of them may

be more easily transformed into pathogenic varieties than others. The two strains subjected to animal passage showed originally all characteristics of typical *streptococcus lacticus*. One, however, fermented mannite, the other did not. Pathogenicity developed in similar ratio in both strains. The fact that Strain 1 retained its spherical shape throughout the experiments while Strain 2 assumed in part the picket-fence form is suggestive. The appearance of picket-fence streptococci accompanied by large numbers of leukocytes in milk, is taken by some sanitarians as indicating mastitis in cows.

The experiments show clearly that great variation is possible in the acid-producing power of the two strains of the *streptococcus lacticus*. The variability depends on at least two factors: The amount of free available oxygen; the influence of certain nutritive substances. If the organism becomes accustomed to albuminous food in the living animal the power to ferment test substances is reduced. How rapidly the original ability to ferment may be re-established and under what conditions raffinose and inulin may be fermented by strains which originally do not ferment them is an open question. The fermentative ability of the original strains was not changed materially after thirty-eight transfers, respectively, in litmus milk.

CONCLUSIONS

Chain formation of the *streptococcus lacticus* is favored by animal passage and by cultivation in media containing blood serum, without the addition of carbohydrate.

The power to hemolyze human and goat's blood may be acquired to some extent by animal passage.

The presence of a capsule could not be demonstrated at any time. A pathogenic streptococcus does not necessarily possess a capsule.

Animal passage develops and increases virulence of the *streptococcus lacticus*, and the virulence develops at a more rapid rate in rabbits than in guinea-pigs.

Acid production in certain test substances is variable. While the amount of acid produced was not materially changed by fifty-eight consecutive transfers through litmus milk, it was materially changed by animal passage.

By animal passage, the amount of acid produced by the original strain progressively decreased, and fermentation of some of the substances was inhibited. Raffinose and inulin, which were not fer-

mented by the original cultures, were attacked with production of relatively small amounts of acid under certain conditions. Moderate oxygen supply seems to favor fermentation of raffinose and inulin. One strain temporarily acquired the ability to ferment mannite.

Presence of free oxygen seems to favor the production of acid. Under anaerobic conditions less acid in proportion was formed than under conditions with free access of oxygen, and under anaerobic conditions a smaller number of test substances was fermented than under aerobic conditions.

The fermentative ability is not changed materially by repeated transfers through litmus milk.

THE BACTERIOLOGY OF APPENDICITIS AND ITS PRODUCTION BY INTRAVENOUS INJECTION OF STREPTOCOCCI AND COLON BACILLI*

EDWARD C. ROSENOW

(From the Memorial Institute for Infectious Diseases, Chicago, Illinois)

(WITH PLATES 12 TO 16)

The causes of appendicitis, exclusive of foreign bodies within the lumen of the appendix or other mechanical factors, are still obscure. The excellent, recent studies of Heyde¹ and others on the fluid contents of the appendix and peritoneum show the important rôle anaerobes probably play, at least as secondary invaders. The histological studies of Aschoff² emphasize the importance of streptococci and demonstrate that infection may pass from the lumen directly into the wall, especially if fecal stones are present. Adrian,³ Kretz,⁴ and Cannon⁵ have observed cases of appendicitis following angina, an observation since repeated many times, and they suggest that appendicitis is a blood infection. Poynton and Payne⁶ have reported appendicitis associated with arthritis in two of thirteen rabbits injected intravenously with the so-called *Str. rheumaticus* and in one of six rabbits injected with a streptococcus isolated from the inflamed appendix in a case of rheumatism. They were unable, however, to produce appendicitis with strains from the throat in the latter case. Ghon and Namba⁷ have shown that spontaneous lesions of the appendix of rabbits occur and that appendicitis rarely develops in pyemia, and they suggest, with Aschoff, that if appendicitis begins commonly as an embolic infection then appendicitis must be due to a specific streptococcus or other organism having an elective affinity for the appendix. I⁸ have observed that the streptococci from rheumatism, especially after animal passage, and also streptococci from other sources, when they have acquired a certain grade of virulence, occasionally produce appendicitis in rabbits on intravenous injection.

* Received for publication January 2, 1915.

1. Beitr. z. klin. Chir., 1911, 76, p. 1.
2. Ergebn. d. inn. Med. u. Kinderh., 1912, 9, p. 1.
3. Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, 7, p. 407.
4. Ztschr. f. Heilk., 1907, 38, p. 2960.
5. Deutsch. Ztschr. f. Chir., 1908, 95, p. 21.
6. Researches on Rheumatism, New York, 1914.
7. Beitr. z. path. Anat. u. z. allg. Path., 1912, 52, p. 130.
8. Jour. Infect. Dis., 1914, 14, p. 61.

As yet, no one has demonstrated that bacteria from a possible focus of infection, as the tonsil, in appendicitis will often localize in the appendices of animals when injected intravenously. It occurred to me that a careful, differential, bacteriological study of the fluids and tissues in and about the appendix and of the tonsils and other foci, with intravenous injections into animals of the isolated strains, might throw light on the question of the development of appendicitis.

TECHNIC

As soon as removed, the appendix was covered with sterile gauze. The cultures were usually made as soon as possible, but, in some instances, the appendix was put in the refrigerator overnight and cultures were made the following morning. Small pieces of the peritoneal coat and mesentery showing inflammation were removed in a sterile manner either with small scissors or scalpel. The appendix was then opened, the contents drawn into a sterile pipette, and a small portion of the wall removed after the mucous membrane had been thoroughly washed in sterile, running water. The tissues were then washed in sterile salt solution and emulsified with 5 c.c. of dextrose broth in a sterile mortar.

Inoculations of similar amounts of the emulsion and pus, or other material, were made on blood agar plates, ascites dextrose agar plates or in tall tubes, on anaerobic blood agar slants, and into tall columns (9-11 cm.) of ascites dextrose broth in tubes or bottles with or without sterile tissue. The fluid from the edematous appendical wall was sometimes inoculated into sterile, undiluted serum. It is important to use tall columns of broth so that the growing bacteria have a wide range of oxygen pressure, especially if selective localization is to be obtained.

The cultures were incubated at 35-37 C. from eighteen to twenty-four hours. They were then centrifugated, the broth poured off, and suspensions made in salt solution in such a way that 1 c.c. of suspension was equivalent to the growth from 15 c.c. of broth. The cultures from the tonsils were made in a similar way. In the tables, unless otherwise indicated, the number of cubic centimeters represents the growth in ascites dextrose broth. Most of the injections were made with mixtures of all the organisms which developed in the primary cultures in ascites dextrose broth and the organisms isolated from the lesions in the rabbits.

The injections were rapidly made in the marginal ear veins of rabbits with an ordinary hypodermic needle. No attention was paid to

the feeding time or character of the food, altho the injections were usually made late in the afternoon some hours after the animals were fed for the day. Control cultures of the suspensions were made just before the injections. This is important, because negative results were found often to be due to the fact that only dead bacteria were injected.

The animals were examined as soon after death as possible. This is also important because after death the appendix is quickly invaded by bacteria from the lumen. A painstaking search for lesions was made. In the case of joints, periarticular hemorrhages and turbidity of the articular fluid were regarded as due to arthritis. In the tables, the number of plus signs (+) roughly indicates the amount of lesion in the different organs. The early lesions were usually localized hemorrhages; the later, often localized edema and necrosis.

The cultures from the appendix and tissues in the animals were made either by emulsifying the tissues in a mortar or by drawing them into a pipette.

Tissues were fixed in 10 percent formalin or Zenker's fluid, stained with hematoxylin and eosin by the Gram-Weigert method. When the colon bacillus was not decolorized completely it could be easily recognized.

REVIEW OF CASES, CULTURE RESULTS, AND ANIMAL EXPERIMENTS

CASE 128.—Recurrent appendicitis and tonsillitis in a boy 12 years old. Present attack of appendicitis not definitely connected with an acute tonsillitis. Appendix, tonsils, and adenoids removed August 21 by Dr. Balfour. The appendix is hyperemic, free from constricting bands and fecal concretions, and contains a small amount of mucopurulent material; a lymph gland the size of a large pea is found at its beginning; the mucous membrane shows marked hyperemia and numerous punctate hemorrhages, especially near the tip, but it is not ulcerated.

Cultures from the lumen of the appendix yield colon bacilli; from the wall, colon bacilli and feebly green-producing streptococci; from the gland, feebly green-producing streptococci; and from the tonsils, feebly hemolysing and green-producing streptococci. The streptococci from the wall and gland are identical and resemble very closely the green-producing streptococcus from the tonsils.

Sections of the appendix show moderate hemorrhagic and leukocytic infiltration, which are most marked in the mucosa, submucosa, and lymph follicles. The mucous membrane shows a few streptococci and bacilli; the deeper layers show a few diplococci.

Intravenous injection of streptococci from the tonsil (Rabbits 686, 689, 692, 693, 702, and 703), mixed cultures of the colon bacillus and streptococcus from the wall of the appendix (Rabbit 705), and pure cultures of the latter (Rabbits 709, 710, 713, and 714) were followed, as shown in Table 3, by lesions in the appendix in all but two of the animals injected (Rabbits 692 and 702), while pure cultures of the colon bacillus failed to produce lesions in the appendix (Rabbits 687 and 704). The streptococcus from the appendix, when passed through ani-

imals, lost its affinity for the appendix and acquired an affinity for the mucous membrane of the stomach and duodenum (Rabbits 719, 720, 724, 728, Dog 131, and Fig 1234).

Rabbit 686.—Injected intravenously on August 23 with the growth from 75 c.c. of ascites dextrose broth of the original culture from the emulsified tonsil.

August 24, seems quite well; chloroformed and examined at once. Multiple hemorrhages of mucous membrane of appendix particularly over base. No hemorrhages in small or large intestine. A few small punctate hemorrhages of mucous membrane of cardiac end of stomach. No other gross lesions. August 25, bile and joint fluid sterile. Pure culture green-producing streptococcus from blood. Sections of the appendix show small hemorrhages just beneath the mucosa.

Rabbit 705.—Injected intravenously on August 26 with the mixed growth from 15 c.c. ascites dextrose tissue broth of streptococcus and colon bacillus from the wall of the appendix.

August 27, found dead. Approximately fifty small punctate hemorrhages in the mucous membrane of the appendix. A few hemorrhages in the right ventricle. No other gross lesions except marked, cloudy swelling of heart and kidney. August 29, large number of colon bacilli and streptococci from the blood and wall of appendix. Sections of the appendix show moderate number of small hemorrhages just beneath the mucosa and in the lymph follicles.

Rabbit 709.—Injected intravenously on August 28 with the growth from 60 c.c. of ascites dextrose broth of the streptococcus from the wall of the appendix.

August 31, seems quite well; chloroformed and examined at once. Large number of small, fading, punctate hemorrhages in the mucous membrane of the appendix surrounded by hyperemia. Joint fluid turbid; no other gross lesions. September 2, joint fluid sterile. A pure culture of distinctly green-producing streptococcus from blood.

Rabbit 719.—Injected intravenously on September 1 with the growth from 45 c.c. of ascites dextrose broth of the streptococcus from the appendix after one animal passage.

September 3, seems ill; chloroformed and examined at once. Four punctate hemorrhages in the duodenum in an area 3 mm. in diameter. The intervening mucous membrane seems swollen and opaque. One ulcer, 3x4 mm., in the stomach 2 cm. from the pyloric ring. The ulceration extends through the mucous membrane, the margins are abrupt, the base hemorrhagic. Similar ulcer, 2x3 mm., in the lesser curvature. Joint fluid very turbid; no other gross lesions. September 4, blood and joint fluid, sterile; bile, large number streptococci; ulcer, forty colonies of streptococci, pure.

CASE 184.—Acute gangrenous appendix in a girl, 17 years old. Present attack began four days ago with acute pain in abdomen which was rather diffuse at first, but later became localized in the right lower quadrant and was associated with nausea and vomiting. She had a similar, but milder, attack one month before. There has been no history of sore throat, but since the last attack she has developed a cough and raises a moderate amount of sputum. On October 31, a gangrenous appendix embedded in a mass of edematous adhesions was removed by Dr. C. B. Davis. On October 31, appendix is much swollen, dark red, and nearly perforated near the base. The lumen contains a moderate amount of bloody pus, without foul odor, and is free from fecal concretions and foreign bodies. The mucous membrane is necrotic throughout.

On November 2, cultures from the lumen yield chiefly colon bacilli and a few streptococci. Blood agar plate cultures from the hemorrhagic peritoneal coat near the tip showed 45 colonies and from the peritoneal coat near the base, 3,600 colonies of a streptococcus, which produces a distinct green and a narrow zone of hemolysis peripheral to the green zone. A pure culture of the same streptococcus is found also in ascites dextrose broth and agar and in the anaerobic cultures. The wall of the appendix shows chiefly this streptococcus but also a moderate number of colonies of the colon bacillus. No fusiform bacilli or spirilla.

Sections from the appendix show marked hemorrhage and leukocytic infiltration throughout the wall, but principally in the submucosa, the lymph follicles, and subperitoneal coat. In many places, the connective tissue and muscle fibers are widely separated by large areas of hemorrhage in which are relatively few leukocytes. Gram stains show moderate number of gram-positive diplococci and short chains. These are found both inside and outside of the leukocytes. The streptococci are most numerous in areas where necrosis and leukocytic infiltration are pronounced. Sections stained by Greenwald's blood stain show fully one-third of the polynuclear leukocytes to be eosinophils. A few bacilli corresponding to colon bacilli are found in the mucous and submucous layers. No thrombosis of vessels can be made out.

On November 2, the tonsils are small, ragged, and a small amount of pus (1 c.c.) is expressed from a deep crypt in the upper pole of the right tonsil. The cultures, on November 3, in ascites dextrose broth of the pus from the tonsil show a large number of short-chained, non-clumping streptococci and what appear to be colon bacilli. The blood-agar plates show chiefly the streptococcus viridans, a few hemolytic streptococci, and colonies resembling colon bacilli.

The tonsil was again examined on November 6 and a similar quantity of pus expressed from the same crypt. November 7, the cultures in ascites dextrose broth, instead of producing a diffuse turbidity containing short chains of streptococci, produce a marked flocculent sediment composed of extremely long chains of streptococci, often in large clumps. The bacilli resembling the colon bacillus are absent. Blood agar plate made directly from the material from the tonsil shows chiefly the streptococcus viridans and a few hemolytic streptococci and staphylococci. A culture from the sputum shows a moderate number of colonies of streptococci, both hemolysing and green-producing, and colon bacilli.

A small amount of pus is again expressed from the crypt on November 24 in the right tonsil and cultures made. The patient is now well and is about to leave the hospital.

The results of the intravenous injection, as shown in Table 4, are as follows: The first culture from the edematous peritoneum (Rabbits 785, 851, 852, 853, 854, 855, 864, 865, and 866) containing a pure culture of streptococci shows a striking affinity for the appendix. The colon bacillus from the appendix showed no special predilection for the appendix (Rabbits 885 and 886). The streptococcus isolated from the appendix of rabbits, injected with the mixed cultures of streptococcus and colon bacillus isolated both from the tonsil and appendix in the patient, show similar, but less striking, lesions; hemorrhages of the stomach and duodenum were more common (Rabbits 862, 863, 868, 869, 870, 877, 878, 879, and 880). Rabbit 883 showed lesions in the appendix, stomach, and gall-bladder. The third animal passage of the streptococcus, originally isolated from the appendix, produced no lesions of the appendix, but showed

an affinity for the gall-bladder and joints instead (Rabbits 875 and 894). Pure cultures of the streptococcus from tonsil forty-eight hours after operation produced marked hemorrhages of the appendix only (Rabbit 890). Pure cultures of the colon bacillus produced only slight hemorrhages of the appendix, duodenum, and small intestines (Rabbits 885 and 886). The elective affinity for the appendix of the mixed cultures of the streptococcus and colon bacillus lasted for nine days (Rabbits 887 and 888), while the pure cultures of the streptococcus from the tonsil had nearly lost it (Rabbit 829), and the one from the appendix had entirely lost it (Rabbit 831).

As the streptococci in the test tube lost the affinity for the appendix, those in the crypt of the patient's tonsil also lost their affinity, because cultures of the pus expressed six and twenty-five days after the operation failed to show the slightest tendency to infect the appendix, but now produced neither lesions nor arthritis, endocarditis, or myocarditis (Rabbits 881 and 882). The recovery of the colon bacillus from the tonsil was unusual and, because the lesions in the appendix with mixtures of the streptococcus and colon bacillus were so striking, it was thought that possibly the streptococcus had acquired this peculiar property from growth in symbiosis with the colon bacillus. The streptococcus from the tonsil in the second culture, which showed no tendency to infect the appendix, was now grown in ascites dextrose broth with the colon bacillus. The mixture was then injected, but no lesions of the appendix developed (Rabbits 891 and 893).

Rabbit 847.—Injected intravenously on November 1 with the growth from 1.5 c.c. ascites dextrose tissue broth inoculated with the emulsion of the appendix and containing streptococcus and colon bacillus.

November 3, seems quite well; chloroformed. No gross lesions except a moderate number of punctate hemorrhages in the cortex of the kidney and three poorly circumscribed areas of infection associated with hyperemia and edema in the mucous membrane of the appendix. Each of these is situated in the more avascular portion of the appendix where the anastomosis of the blood vessels occurs.

November 5, cultures from the areas of infection in the appendix show a large number of streptococci and only a few colonies of colon bacilli, while the blood yields an almost pure culture of streptococcus, a few colonies of colon bacilli, and, in addition, the shake cultures in ascites dextrose agar yield the bacillus welchii.

Rabbit 857.—Injected intravenously on November 3 with the growth from 15 c.c. of ascites dextrose broth from the tonsil containing streptococcus and colon bacillus.

Found dead on November 4. The appendix is almost black with a large number of hemorrhages, chiefly in the submucosa and subperitoneal coat. Moderate number of smaller punctate hemorrhages at the ileocecal valve, the lymph follicles of the small intestines, a few in the mucous membrane of the duodenum, but none in the stomach, kidneys, adrenals, heart, brain, liver, spleen, eyes, or testicles. The lungs show a moderate number of small hemorrhages. Joint fluid clear.

November 6, cultures from the wall of the appendix wall yield many green colonies of streptococci and a few colonies of colon bacilli; from the blood, moderate number of green-producing streptococci and a few colon bacilli.

Sections of the appendix show marked hemorrhage chiefly in the spaces between the lymph follicles, but also in the latter. The nuclei of the fixed

cells here stain poorly and large numbers of streptococci and a few bacilli are present.

Rabbit 860.—Injected intravenously on November 3, with the growth from 30 c.c. of ascites dextrose broth inoculated with the pus obtained from the tonsil forty-eight hours after the operation and containing streptococci and colon bacilli.

November 4, found dead. Marked hemorrhages of the appendix; desquamation of the mucous membrane; the lumen free from fecal material. Moderate number of hemorrhages at ileocecal junction and a number of small areas in the lymphoid structures of the small intestines. The lymph gland at the root of the appendix is hemorrhagic, while those in the mesentery appear normal. No hemorrhages in stomach or duodenum. Gall-bladder, pancreas, heart valves, skeletal muscles, myocardium, kidneys, adrenals, eyes, brain, and thyroid show no hemorrhages. Liver is gray and opaque.

On November 5 the blood agar plates from the blood show a moderate number of green-producing streptococci and colon bacilli. The peritoneal coat overlying the appendix shows eight colonies of streptococci and one colony of colon bacilli. Sections show marked hemorrhage in the submucosa and in the lymph follicles. The nuclei of the tissue cells are fragmented and stain poorly, while stains for bacteria show many streptococci and some bacilli resembling colon bacilli.

Rabbit 862.—Injected on November 4 with the growth from 45 c.c. ascites dextrose broth of the streptococcus isolated from the appendix in Rabbit 847, which was injected with the mixture of the streptococcus and colon bacillus from the lumen of the appendix.

November 5, found dead, body warm. Marked hemorrhages in the appendix and in two Peyer's patches. Moderate number of smaller subperitoneal and submucous hemorrhages throughout the small and large intestine, most marked at the ileocecal junction. The lumen of the appendix is free from fecal material except for 1 cm., and contains a large amount of brownish mucus in which is a great quantity of desquamated material. The contents of the small intestines give a strong Weber test for blood. One large subcutaneous hemorrhage over right shoulder and a few hemorrhages in the heart valve; no other gross lesions.

On November 6 blood agar plates and ascites dextrose agar plates from the emulsion of the wall of the appendix show countless numbers of slightly green-producing streptococci and only forty colonies of colon bacilli. Cultures in ascites dextrose broth give similar results. The blood shows a pure culture of streptococcus. The joint fluid shows a few colonies of streptococci. Sections of the appendix show the hemorrhages are most marked in the lymph follicles, submucosa, and subperitoneum. The mucous membrane over these areas is absent. The nuclei of the fixed cells, in and adjacent to the areas which contain large numbers of streptococci, stain poorly. In several areas, the capillaries adjacent to areas of hemorrhage are plugged with streptococci and leukocytes.

Rabbit 865.—Injected intravenously on November 4 with the growth from 30 c.c. of the streptococcus isolated in pure form from the peritoneal coat of the appendix, now in the second culture.

November 6, seems quite well; chloroformed. Moderate number of fading hemorrhages in the appendix. No other gross lesions.

On November 10, cultures from the blood yield pure culture of green-producing streptococcus, from the joint fluid a few small slightly green colonies of streptococci.

Rabbit 880.—Injected intravenously November 6 with the growth from 15 c.c. ascites dextrose broth of the streptococcus isolated from the tonsil.

November 7, found dead, body warm. Appendix shows one large subperitoneal hemorrhage and numerous punctate hemorrhages in the submucosa; it contains a large amount of very turbid mucus but no fecal material. Moderate number of small punctate hemorrhages throughout the small intestine, particularly of Peyer's patches. Marked hemorrhages in the duodenum and cardiac end of stomach. The hemorrhages in the duodenum are distributed in a circular area around the ampulla of Vater. Joint fluids clear, no hemorrhages in skin. No other gross lesions.

On November 10 cultures from appendix yield many colonies of green-producing streptococci and a few colon bacilli; the blood yields a pure culture of streptococcus. Sections of the appendix show marked hemorrhage and necrosis of the submucosa and lymph follicles.

Rabbit 883.—Injected intravenously on November 7 with the growth from 45 c.c. of ascites dextrose broth of the streptococcus isolated from the blood in Rabbit 858, which was injected with the streptococcus isolated from the human appendix.

November 8, very ill; chloroformed. Moderate number of punctate hemorrhages in the appendix; the lumen contains a large amount of turbid, flaky mucus, but no fecal material. The lymph glands draining the appendix are hemorrhagic. Marked hemorrhages in the mucous membrane in the stomach with beginning ulceration; a few small hemorrhages in the duodenum; six subperitoneal hemorrhages in the gall-bladder, the largest, which is at the fundus, is surrounded by an edematous area of infection. No other gross lesions. Joint fluids clear.

On November 10 cultures from blood and area of infection in gall-bladder show large number of green-producing streptococci; joint fluid gives a few, while the bile is sterile.

CASE 190.—Acute gangrenous appendicitis in a man, 34 years of age. No history of any associated infection of the throat. On November 9, four days after the symptoms began, a swollen, gangrenous appendix was removed by Dr. Lewis. The lumen is free from fecal material, but contains a rather large amount of bloody pus without foul odor. Smears show a large number of gram-staining diplococci and bacilli resembling colon or fusiform bacilli.

On November 10 cultures on blood agar and ascites dextrose agar plates of the emulsion from the wall show mostly hemolytic colon bacilli and a few grayish colonies of a streptococcus which do not affect the blood in the plates. The peritoneal coat is sterile.

In animal experiments the intravenous injection of the bacteria directly from the emulsion of the appendix, chiefly streptococci, mixed aerobic cultures of colon bacilli and streptococci, and anaerobic cultures of these, containing in addition fusiform bacilli, produced lesions in the appendix almost exclusively in all of five rabbits. The organisms injected (except the fusiform bacillus) were demonstrated in the appendix either in sections or by cultures in the animals after death.

CASE 191.—Typical acute appendicitis in a woman, 45 years old, who has subject to tonsillitis and appendicitis, but this attack began without associated tonsillitis or other infection. A much swollen, almost black, perforated appendix was removed November 10 by Dr. Davis, thirty-six hours after the attack began. The patient died of general peritonitis three days later. The lumen

contains a small amount of bloody, foul-smelling pus and two fecal concretions. The mucous membrane is gangrenous and opposite the concretions the wall is perforated. The mesentery contains a large amount of fat and numerous hemorrhages. Smears from the fluid in the mesentery show gram-staining cocci and diplococci and a few gram-negative bacilli. The pus from the appendix shows gram-negative bacilli, resembling colon bacilli and fusiform bacilli, and a moderate number of gram-staining cocci.

On November 12 cultures from the pus within the appendix show colon bacilli, staphylococci, and an unidentified, small gram-negative bacillus; the wall of the appendix gives many gas bacilli, a few streptococci, a few colon bacilli, fusiform bacilli, and staphylococci; the peritoneal coat and edematous mesentery, many moderately hemolysing streptococci, a few gas bacilli and fusiform bacilli, and the staphylococcus aureus (Table 1). The cultures from the crypts of the tonsils show a predominating number of hemolytic streptococci, but also the streptococcus viridans and a moderate number of the staphylococcus aureus. The blood agar plates made from the ascites dextrose broth cultures inoculated with the material from the tonsils show a moderate number of colonies of streptococci indistinguishable from those isolated from the edematous mesentery.

Sections of the appendix show marked hemorrhage, leukocytic infiltration, and in areas necrosis in all the layers. The leukocytic infiltration is most marked surrounding the areas of necrosis in lymphoid tissue in the submucosa and in the peritoneal coat. The mucous membrane is absent. Gram-Weigert stains show many diplococci in chains together with single cocci, and, in addition, the superficial layers show a large number of bacilli resembling fusiform bacilli.

In the animal experiments the intravenous injection of the original cultures from the wall of the appendix and edematous fluid, containing mixtures of colon bacilli, streptococci, and a few staphylococci, failed to produce lesions in the appendix in three rabbits. The pure culture of slightly hemolysing streptococcus in the second culture isolated from the edematous mesentery also failed to produce appendicitis, but, instead, produced suppurative arthritis in four rabbits. On the other hand, the original culture from the tonsils, containing streptococci and staphylococci, produced distinct lesions in the appendix in two rabbits. The hemorrhages in the appendix seem to have been due in both instances to the staphylococcus, as shown by the cultures from the appendix. (See Rabbit 911.)

Rabbit 911.—Injected intravenously on November 12 with the growth from 45 c.c. of ascites dextrose broth inoculated with the material from the tonsils containing streptococcus and staphylococcus.

November 13, found dead. Numerous punctate hemorrhages in the mucous membrane and subperitoneum of the appendix. Joint fluid slightly turbid and moderate number of hemorrhages about knee joints. No other gross lesions.

November 14, blood agar plates, inoculated with the bloody fluid in the wall of the appendix, show large numbers of hemolysing colonies of staphylococci and a few streptococci; from the blood and joints, a large number of slightly hemolysing streptococci and a few staphylococci; from a bile, a few colonies of hemolysing staphylococci.

Rabbit 916.—Injected intravenously on November 13 with the growth from 10 c.c. of serum tissue broth of slightly hemolysing streptococci isolated from the edematous mesentery (second culture).

November 14, seems sick; chloroformed. No gross lesions except turbid joint fluid and periarticular hemorrhages.

November 16, blood agar plates inoculated with the blood and joint fluid show fifty colonies and four colonies, respectively, of a feebly hemolytic streptococcus.

CASE 199.—Typical, acute appendicitis in a girl, 13 years old. The attack came on late in the course of a severe Vincent's angina with cervical adenitis. Smears from the throat (Dr. Moody) showed a large number of streptococci and fusiform bacilli. A gangrenous, foul smelling appendix was removed on November 15 by Dr. Halstead, seventy-two hours after the symptoms began. The appendix is much swollen, edematous, and dark red. The tissues are friable. The lumen contains one fecal concretion about the size of a navy-bean and a small amount of foul smelling, bloody pus. Mucous membrane is necrotic and ulcerated in places. Smears of the pus from the emulsion of the wall of the appendix and of the edematous peritoneal fluid show what appear to be streptococci, fusiform bacilli, and colon bacilli.

On November 17, the cultures in ascites dextrose broth of the emulsions from the wall, peritoneal coat, and edematous mesentery show colon bacilli, streptococci, fusiform bacilli, and spirilla. The cultures have a characteristic, foul odor. Blood agar and ascites dextrose agar plates show green-producing streptococci and colon bacilli from all. The proportion of streptococcus colonies increases as the cultures are made from the contents outward. The blood agar plate cultures from the tonsils show approximately an equal number of hemolyzing and green-producing streptococci and a few staphylococci. Anaerobic cultures in ascites dextrose broth and on blood agar slants show streptococci in rather long chains and clumps and large numbers of fusiform bacilli.

Sections of the appendix show marked hemorrhages and leukocytic infiltration, particularly marked in the submucosa, subperitoneum, and lymphoid structures, while the muscular layer is relatively free. In many areas, the nuclei fail to stain. Gram-Weigert stains show large numbers of diplococci and chains, which appear in pure culture in the deeper layers and surrounding the areas of necrosis, while in the more superficial layers and in the center of the necrotic areas a large number of fusiform bacilli are also found.

In the animal experiments the intravenous injection of the primary aerobic and anaerobic cultures from the tonsil in ascites dextrose broth, containing streptococci, was followed by small hemorrhages in the appendices in all of the rabbits injected. In the second culture, the appendix was not affected but arthritis developed. The anaerobic cultures from the tonsil in ascites dextrose broth, containing streptococci and fusiform bacilli, produced lesions in the appendix and arthritis, while the anaerobic culture on blood agar produced only arthritis and hemorrhages in muscles. The primary aerobic cultures of the edematous mesentery and appendix, containing streptococci and colon bacilli, produced slight lesions of the appendices of two animals. The cultures, in which sterile tissue was added and which contained the fusiform bacillus in addition, and also those containing streptococcus and fusiform bacillus without colon bacillus produced marked lesions of the appendix in all but one of the rabbits. In the second culture, the affinity for the appendix was lost.

Rabbit 928.—Injected intravenously on November 17 with the growth from 30 c.c. ascites dextrose tissue broth containing streptococci, colon bacilli, and fusiform bacilli.

On November 18 animal was very ill; chloroformed, and examined at once. Moderate number of subperitoneal, circumscribed hemorrhages in the appendix

Two small hemorrhages in the gall-bladder, one small hemorrhage in the ileum, a few under the skin in the muscles and kidney. Joint fluid clear.

November 19, aerobic blood agar plates from the blood show thirty-two colonies of colon bacilli and six colonies of a distinctly green-producing streptococcus. Hemorrhagic fluid under the peritoneum, in the appendix, from the skin and the joint fluid show no growth. One small hemorrhagic area in the appendix ground up in a mortar shows five colonies of green-producing streptococci, while another shows both streptococci and colon bacilli. The anaerobic cultures from the hemorrhagic area in the skin remain sterile while those from the lesions in the appendix give colon bacilli, fusiform bacilli, and streptococci.

Sections of the appendix show marked extravasation of red blood corpuscles and areas where the cells stain poorly. These contain a moderate number of streptococci and bacilli. Some of the latter resemble fusiform bacilli.

Rabbit 932.—Injected intravenously on November 18 with growth from 8 c.c. ascites dextrose tissue broth from the mesentery, containing streptococci, colon bacilli, and fusiform bacilli.

November 19, found dead. Large number of small punctate hemorrhages and four larger hemorrhages in the mucous membrane of the appendix; a few in the small intestines and at the ileocecal valve; two subperitoneal hemorrhages over the gall-bladder; and a few subpleural hemorrhages.

On November 21 cultures from the blood show almost a pure growth of green-producing streptococci and a few colonies of colon bacilli. The bile shows colon bacilli only, the hemorrhagic area in the appendix chiefly green-producing streptococci but some colon bacilli also. The fusiform bacillus is not found in the anaerobic cultures.

Rabbit 937.—Injected intravenously on November 19 with the growth from one anaerobic blood agar slant, inoculated with the emulsion from the mesentery containing streptococci and fusiform bacilli.

November 20, found dead. Large number of subperitoneal and submucous hemorrhages in the appendix, an occasional subperitoneal hemorrhage over the small intestine, and a large number of very small hemorrhages in the mucous membrane of the duodenum 2 cm. from the pyloric ring; a few small hemorrhages in the limbus of the left eye. No other gross lesions.

Smears from the emulsion of the peritoneal coat and hemorrhage in duodenum are negative; of the hemorrhagic wall of the appendix, show gram-positive and gram-negative bacilli and diplococci; from the hemorrhage in the eye, fusiform bacilli and diplococci.

November 22, cultures from the blood show streptococci, fusiform bacilli, and colon bacilli; from the duodenum, colon bacilli and streptococci; from the appendix, streptococci, fusiform bacilli, and colon bacilli; from the hemorrhage in the eye, colon bacilli and fusiform bacilli.

Sections of the appendix show a number of large and small hemorrhages and areas of necrosis in the connective tissue between and in the lymph follicles and submucosa. A large number of streptococci and fusiform bacilli are found in these areas. Sections of the duodenum show a moderate number of streptococci but no fusiform bacilli.

CASE 203.—A mild attack of appendicitis in a man. 19 years old. No history of tonsillitis or other associated infection; has not felt just right for two weeks and has lost twenty pounds in weight. A hyperemic and somewhat swollen appendix was removed on November 21, by Dr. Bevan, one week after the acute symptoms began. The lumen is free from fecal concretions and constricting bands and contains a small amount of bloody pus without odor; the

mucous membrane is hemorrhagic over two small areas and markedly congested everywhere.

On November 24, the cultures from the lumen, the wall, and peritoneal surface of the appendix show large numbers of hemolytic colon bacilli and a streptococcus which produces small, grayish, glistening, non-adherent colonies on blood agar plates, the peritoneal coat giving in addition a diphtheroid bacillus. Cultures from the right tonsil, which was swollen, showed the usual hemolysing and green-producing streptococci and a few colonies of staphylococci. Sections of the appendix show slight hemorrhages in the lymph follicles, submucosa, and just beneath the peritoneum. There are little leukocytic infiltration and no bacteria.

In the animal experiments the intravenous injection of the colon bacillus isolated from the appendiceal wall showed a pronounced affinity for the appendix and produced severe submucous and subperitoneal hemorrhages in two rabbits together with similar, but much less marked, lesions in the lymphoid structures of the intestines, and a few hemorrhages in the heart and skeletal muscles. In the second culture, this affinity was already much less marked (five rabbits), while in the fourth culture, twelve days later, the affinity had entirely disappeared. The mixture of colon bacilli and streptococci isolated from the peritoneal coat showed hemorrhages in the appendix and heart valves in one rabbit while the diphtheroid bacillus from the peritoneal coat failed to produce lesions anywhere in two rabbits. The mixed culture of the hemolytic and green-producing streptococci from the tonsil produced small hemorrhages in the appendix and arthritis in one rabbit, while the other showed hemorrhages in the tricuspid valve.

CASE 205.—Acute appendicitis in a man, 27 years old. No history of a sore throat. On November 25, fifty hours after the attack began, a markedly hyperemic and edematous appendix was removed by Dr. Phemister. A thick deposit of fibrin is found along the base and the mesentery; the lumen is free from fecal material and contains a small amount of bloody, odorless pus; the mucous membrane is hemorrhagic and in areas necrotic but there is no perforation. Smears from the pus in the appendix show a large number of gram-negative bacilli while those of the fibrin show streptococci only. The patient had fever for a number of days following the operation but made a good recovery.

Cultures from the pus show 5,400 colonies of colon bacilli; the emulsion of the wall, 680 colonies of a distinctly green-producing streptococcus and 14 colonies of colon bacilli; the peritoneal coat, 2,200 colonies of streptococci and 9 colonies of colon bacilli; and the fibrin, countless numbers of streptococci and 65 colonies of colon bacilli.

November 28, cultures which were made (November 27) from the pus expressed from the pockets in the tonsils and from the reddened, infected gums around a badly decayed, loose tooth yield chiefly short-chained streptococci in ascites dextrose broth; on blood agar, there is a great preponderance of green-producing colonies of streptococci. Sections of the appendix show marked hemorrhage, particularly just outside the circular muscle fibers. The mucous membrane and peritoneal coat are infiltrated with red blood corpuscles and leukocytes. Gram-Weigert stains for bacteria show diplococci and streptococci which are most numerous in the peritoneal coat and adjacent to the zone of hemorrhage. In the mucous membrane, a few bacilli are found.

In the animal experiments the intravenous injection of the streptococci in ascites dextrose broth from the tonsils and tooth and from the peritoneal coat, as well as the mixture of the colon bacillus and streptococcus from the wall

of the appendix, produced hemorrhages in the appendix in all but one of seven rabbits. The colon bacillus alone produced hemorrhages in the intestine but not in the appendix.

CASE 210.—Acute appendicitis in woman 19 years of age, following an attack of sore throat. On November 30, twelve hours after the symptoms of appendicitis began, a hyperemic and swollen appendix, 8 cm. in length, was removed by Dr. C. B. Davis. The lumen contains a moderate amount of bloody pus; there are no concretions or constrictions; the mucous membrane is hemorrhagic throughout. Smears from the pus show a large number of gram-staining diplococci and short chains and a few bacilli resembling colon and fusiform bacilli.

The cultures from the pus yield streptococci, colon bacilli, the bacillus welchii, and an unidentified bacillus; from the wall, colon bacilli, streptococci, the bacillus welchii and a diphtheroid-like streptococcus. The pus from the lumen in human serum gives many streptococci and a few fusiform and colon bacilli; in horse serum, there develops an almost pure culture of streptococci. Blood agar plates show that the streptococci from all three places in the appendix produce green colonies.

December 2, the cultures from the small amount of pus, expressed from the inflamed tonsils, in ascites dextrose tissue broth show short-chained streptococci and fusiform bacilli. Blood agar plates show a large preponderance of green-producing streptococci. Sections of the appendix show marked hemorrhage and leukocytic infiltration, particularly of the mucosa, submucosa, and the lymph follicles.

In the animal experiments the original cultures in ascites dextrose broth, containing a mixture of colon bacilli and streptococci from the lumen and wall of the appendix, the pure culture of the streptococcus from the peritoneal coat and the mixture of streptococcus and fusiform bacillus from the tonsil produced pronounced lesions in the appendix in four of six rabbits. The aerobic cultures from the tonsil, containing streptococci only, produced no lesions. The streptococcus from the lumen of the appendix, after one animal passage, produced marked hemorrhages in the gall-bladder, but no other lesions.

CASE 212.—Acute appendicitis in a medical student, 22 years old. Symptoms referable to the appendix began on the sixth day of an attack of severe tonsillitis, from which, however, he had recovered sufficiently to attend class late in the afternoon. The symptoms of appendicitis began that night. On December 3, twelve hours later, a markedly edematous and inflamed appendix was removed by Dr. C. B. Davis. The swelling is most marked near the tip opposite a cluster of fecal concretions; there is a rather thick fibrinous deposit; the mucous membrane opposite the concretions is markedly hemorrhagic and necrotic while the rest shows numerous small hemorrhages; the pus in the appendix is odorless.

The aerobic cultures from the pus within the appendix show a large number of the bacillus pyocyaneus, the bacillus coli, and streptococci, while the emulsion of a portion of the wall, from the peritoneal coat and edematous mesentery, after a thorough washing, shows a large number of the bacillus pyocyaneus and streptococci. The anaerobic cultures on blood agar have no odor and do not contain fusiform bacilli. The cultures from the tonsil, made December 3 at the time of the operation, show mostly green-producing streptococci and probably pneumococci and a few hemolytic streptococci. The streptococcus from the appendix and the green-producing streptococcus from the tonsil appear identical.

December 10, tonsils are somewhat red but do not contain cheesy material or pus in crypts. December 11, blood agar plates from the tonsil show almost pure culture of very small hemolytic colonies of streptococci and short chains in ascites dextrose broth.

Sections of appendix, opposite the fecal concretions, show marked leukocytic infiltration especially of the lymphoid tissue, submucosa, and peritoneal coat. The latter is covered with a layer of fibrin containing bacilli and streptococci. The mucous membrane is absent. Bacilli and streptococci are found throughout the wall. The latter appear in masses especially in the subperitoneal layer, where there are also three thrombosed blood vessels in one of which a few gram-positive diplococci can be seen (see photomicrographs).

In the animal experiments the intravenous injection of the primary aerobic culture from the tonsil, made at the time of the operation, and the second culture of the streptococcus from the edematous mesentery produced marked hemorrhages in the appendix in Rabbits 949, 992, and 993. The anaerobic culture of the former and the third subculture of the latter entirely failed to produce lesions in the appendix. The anaerobic cultures from the tonsil produced arthritis. The pure culture of the bacillus pyocyaneus showed no predilection for the appendix, but produced many small punctate hemorrhages throughout the intestinal tract and in the lung. The streptococci from the tonsil seven days later failed to produce appendicitis or other lesions (Rabbits 991 and 999).

RESULTS OF THE CULTURES

Cultures from the appendix have been made in fourteen cases of acute appendicitis and six cases of chronic appendicitis (Table 1).

Fecal concretions were present in seven; in the rest, no local, mechanical factors could be made out. Streptococci, usually in predominating numbers, were isolated from the tissues of the appendix in seventeen cases. The colon bacillus was found in pure cultures in the pus within the lumen in six cases and in predominating numbers, but with streptococci or other organisms in the rest. The results of the cultures from the wall, after thorough washing, showed that here the chief bacteria were streptococci. The fusiform bacillus was isolated from the wall of the appendix in three cases (Cases 191, 199, and 96); other anaerobic bacilli and spirilla were found in some cases, but were not identified; the bacillus welchii in two; a diphtheroid bacillus in two; the staphylococcus aureus in one; the bacillus pyocyaneus in one, and unidentified cocci and bacilli in two. The strains of streptococci which were found to have an affinity for the appendix formed short chains, much acid and a diffuse turbidity in ascites dextrose broth, but no clumps and, with but two exceptions, produced a moderate amount of green on blood agar plates. After animal passage they produced more green on blood agar and became more like pneumococci, several strains acquiring capsules. They were larger than the streptococcus

viridans obtained from the blood in chronic endocarditis and the colonies, with one exception, were non-adherent. In a number of cases, two types of non-hemolyzing colonies were obtained; one producing green colonies, and the other non-adherent, small, grayish colonies (*Str. fecalis*). The latter, as well as the strains producing hemolysis, showed no special affinity for the animal appendix.

In the peritoneal coat and mesentery there were less colon bacilli in proportion to streptococci than in the other coats, and sometimes they were entirely absent. Thus, the cultures from the contents of the appendix of Case 205 showed 3,400 colonies of colon bacilli, pure; the wall, 680 colonies of streptococci and 14 colonies of colon bacilli; the peritoneal coat, 2,200 colonies of streptococci and 9 colonies of colon bacilli; while the edematous mesentery and fibrin showed countless numbers of streptococci and 65 colonies of colon bacilli.

These results are in accord with the sections and indicate that reliable differential cultures can be made of the various tissues in appendicitis. This is further borne out by the fact that the peritoneal coat in four cases remained sterile when the more superficial layers contained organisms, and that cultures remained sterile in three normal appendices, in one of six appendices with chronic appendicitis, and in three obliterated appendices.

The character of the bacterial flora of the tonsils in the different cases was not characteristic. Streptococci were found in all; in nearly all, the green-producing variety predominated. The staphylococcus aureus was found in rather large numbers in three; fusiform bacilli were in large numbers in two; and the colon bacillus was found in one.

MICROSCOPIC ANATOMY OF THE APPENDIX

The areas of hemorrhage which are found at the end of twenty-four hours are usually in the submucosa, lymph follicles, and subperitoneal region. At this time there is not much leukocytic infiltration. In five of the appendices from young individuals in which the lesions were relatively slight, the distribution of the hemorrhages and other lesions were strikingly similar. In the experimental lesions, the hemorrhages usually had started to fade and the mucous membrane might be eroded at the end of forty-eight hours. The ulceration of the mucous membrane usually begins directly over the edematous lymph follicles with necrosis and leukocytic infiltration. Here, the nuclei of the fixed tissue cells fail to take the stain, or are fragmented and granular. It is in

these areas, as well as in the areas of hemorrhage, that masses of the injected streptococci are found and where the adjacent normal tissues are free from bacteria. In many instances in which mixtures of streptococci and colon bacilli were injected, the former were usually found in predominating numbers and, in some instances, to the exclusion of the latter. Similar conditions have been found in a number of human appendices. In some instances, capillaries in the appendices of rabbits adjacent to an area of hemorrhage have been found plugged with streptococci (see plates). The organisms found have been shown to be alive in many instances. Very large numbers may be found in the lesions eighteen hours after injection when control cultures of the uninvolved portions of the appendix, other normal tissues, and the blood were sterile or contained only few organisms. Moreover, sections of parts in which no macroscopic evidence of lesions could be made out usually failed to show either lesions or bacteria.

Owing to the larger size of the lumen of the appendix, strangulation, with its consequences, has not been observed. In several instances, however, the lesions were so pronounced that the lesions appeared almost gangrenous in twenty-four and forty-eight hours after injection. Following the injection, the hemorrhages and other lesions showed no tendency to localize around chronic coccidial lesions.

RESULTS OF INTRAVENOUS INJECTIONS

A summary of the results from intravenous injection in rabbits of the isolated strains is given in Table 2.

The elective affinity for the appendix of the streptococci in pure culture or in mixture with fusiform bacilli, isolated both from the tonsils at the time of, or soon after, the attack and from the appendix, is striking. Thus, the tonsillar strains produced appendicitis in nineteen of twenty-nine, the appendix strains in twenty-two of thirty, a total of forty-one of fifty-nine. After cultivation on artificial media for a short time the elective affinity is soon lost, and strains isolated from the tonsils some time after appendectomy also appear without elective affinity.

The results following injection of mixtures of streptococci and colon bacilli are similar, altho the tendency to produce lesions in the intestine and gall-bladder is much greater. Thus, of thirty-one rabbits injected, twenty-six showed lesions in the appendix, four of the gall-bladder and thirteen of the intestines, in contrast to one and four,

respectively, of fifty-nine rabbits injected with the streptococcus only. On the other hand, the tendency to produce lesions in the joints and heart valves by the streptococcus, when injected with the colon bacillus, is strikingly less. Arthritis occurred only in two and endocarditis in

TABLE 1
SUMMARY OF BACTERIOLOGICAL STUDY OF APPENDICITIS

Case	Age	Operator	Diagnosis	Duration of Symptoms Before Operation	Organisms Isolated
					Contents of Appendix
128	12	D. C. Balfour	Recurrent appendicitis and tonsillitis	3 days	Colon bacilli
182	12	C. B. Davis...	Acute appendicitis	12 hours	Colon bacillus and streptococcus
184	17	C. B. Davis...	Acute gangrenous appendicitis	4 days	Chiefly colon bacilli and a few streptococci
190	34	D. D. Lewis...	Acute gangrenous appendicitis	4 days	Colon bacillus and streptococcus
191	45	C. B. Davis...	Acute gangrenous appendicitis with perforation	2 days	Colon bacillus, a small, unidentified, gram-negative bacillus, and staphylococcus
199	13	A. E. Halstead	Acute gangrenous fetid appendicitis and Vincent's angina	2 days	Colon bacillus and a few streptococci
203	19	A. D. Bevan...	Acute appendicitis	7 days	Colon bacillus and streptococcus
205	27	D. B. Phemister	Acute appendicitis	2 days	3,400 colonies of colon bacillus
210	19	C. B. Davis...	Acute appendicitis	12 hours	Streptococcus, colon bacillus, B. welchii, and an unidentified coccus
211	19	C. B. Davis...	Acute (mild) appendicitis...	2 days	Colon bacillus and B. welchii
212	22	C. B. Davis...	Acute appendicitis	12 hours	B. pyocyaneus, colon bacillus, and streptococcus
96	12	W. J. Mayo...	Acute recurring appendicitis and tonsillitis	12 hours	Colon bacillus
84	36	D. C. Balfour	Acute gangrenous, perforating appendicitis	2 days	Colon bacillus and streptococcus
73	50	E. S. Judd....	Chronic appendicitis and cholecystitis	Colon bacillus
72	..	E. S. Judd....	Chronic appendicitis
75	..	C. H. Mayo..	Chronic appendicitis
58	Chronic cholecystitis appendicitis obliterans	Colon bacillus
59	Chronic cholecystitis, chronic appendicitis

one rabbit of thirty-one, whereas in thirty rabbits injected with streptococci one arthritis occurred in seven and endocarditis in six. These results are in entire accord with those of injections of pure cultures of the colon bacillus. Of eleven rabbits injected, six showed lesions in the appendix and six in the intestines.

The elective affinity for the appendix also disappears after one to three animal passages, producing now frequently less-marked lesions in the appendix, but more often lesions in the stomach, duodenum, and gall-bladder. Thus, of twenty rabbits injected only ten showed slight

TABLE 1.—Continued
SUMMARY OF BACTERIOLOGICAL STUDY, OF APPENDICITIS

Organisms Isolated			Remarks
Wall of Appendix	Peritoneal Coat	Edematous Mesentery	
Colon bacilli and green-producing streptococci	Sterile	Gland at base of appendix yields green-producing streptococcus only
Streptococcus and colon bacillus	Sterile		
Chiefly streptococci, also a few colonies of colon bacilli	3,600 colonies streptococci		
Colon bacillus, aerobic and anaerobic streptococci, and fusiform bacilli	Sterile		Death 3 days after operation
B. welchii, a few streptococci, colon bacilli, fusiform bacilli, and staphylococci	Many streptococci, a few B. welchii, fusiform bacilli, and staphylococci	Many streptococci, a few B. welchii, a few colon bacilli, and a moderate number of staphylococci	
Colon bacillus, streptococci, fusiform bacilli, and spirilla	Colon bacillus, streptococcus, fusiform bacillus, and spirilla	Colon bacillus, streptococcus, fusiform bacillus, and spirilla	
Streptococcus and colon bacillus	Streptococcus and bacillus		Smears from edematous fluid show colon bacillus, streptococcus, large numbers of fusiform bacilli
680 colonies of streptococci and 14 colonies of colon bacillus	2,200 colonies streptococcus and 9 colonies colon bacillus	Fibrin, countless numbers of streptococci and 65 colonies of B. coli	
Colon bacillus, streptococcus, B. welchii, and diphtheroid-like bacillus	Diphtheroid-like bacillus		
Streptococcus, colon bacillus, and B. welchii	Sterile		Edematous fluid in a serum culture yielded a pure culture of streptococcus
B. pyocyaneus and streptococcus	B. pyocyaneus and streptococcus	Streptococcus and countless number of colonies of B. pyocyaneus	
Streptococcus and fusiform bacillus			
Streptococcus and colon bacillus			Interim operation, appendix thickened Symptoms referable to stomach. Appendix thickened
Streptococcus and colon bacillus	Streptococcus	
Sterile	
Streptococcus and colon bacillus			

lesions in the appendix, ten hemorrhages in the stomach or duodenum, seven ulcers, and eight lesions in the gall-bladder. This is in sharp contrast to the result of immediate injections of the strains from the tonsil at the time of the attack and the appendix, when lesions in the stomach and duodenum were found only seven times and in the gall-bladder only once in fifty-nine rabbits.

TABLE 2

RESULTS FOLLOWING INTRAVENOUS INJECTIONS

Strains Injected	Number of Animals Injected	Number of Times Lesions Were Found in											
		Appendix	Stomach and Duodenum		Gall Bladder	Pancreas	Joints	Endo- cardium	Myocar- dium	Mus- cles	Kid- ney	Intes- tines	
			Hemor- rhages	Ul- cers									
Streptococci in pure culture, or in mixture with fusiform bacilli, soon after isolation, from tonsil at time of attack	29	19	4	0	0	0	10	7	4	5	0	2	
Streptococci in pure culture, or in mixture with fusiform bacilli, from the appendix soon after isolation	30	22	2	1	1	..	7	6	1	2	..	2	
Streptococci from tonsil and from the appendix, after cultivation on artificial media for a short time, and from the tonsils some time subsequent to appendectomy	22	3	5	4	1	0	8	5	3	6	0	0	
Streptococci and colon bacilli in mixed cultures from the tonsil or appendix soon after isolation	31	26	6	0	4	0	2	1	3	3	1	13	
Streptococci from the tonsil or appendix after one or three animal passages	20	10	10	7	8	0	8	4	4	5	2	4	
Pure cultures of the colon bacillus from the appendix, soon after isolation	11	6	3	0	0	0	0	1	2	2	0	6	
Total	143	86	30	12	14	0	36	24	17	23	3	27	

The relatively large number of times arthritis developed after injection of the streptococcus was due no doubt in part to mixtures of the hemolytic streptococcus and green-producing streptococcus from the tonsils and to the slightly hemolyzing strain which was found in the appendix in one of the cases (Case 191).

At times, it was possible to isolate from the rabbits after injection of mixtures green-producing streptococci from the appendix and hemolyzing streptococci from the joint fluid. The streptococci from the appendix, as well as from the tonsil, which show affinity for the appendix are of a relatively low grade of virulence. They tend to disappear from the circulation unless very large doses are given, thereby affording opportunity to study their relation to various lesions produced by making cultures from the tissues involved. In Case 191, which died of peritonitis on the third day, moderately hemolyzing and relatively virulent streptococci were found, which on injection showed no affinity for the appendix, but a marked affinity for the joints. The streptococci isolated from the wall of the appendix in the three cases of chronic appendicitis and cholecystitis, which were identical with the strains from the gall-bladder in two of the cases, showed a tendency to infect simultaneously appendix, stomach, and gall-bladder when injected into rabbits. Thus, of eight rabbits injected, three showed lesions in the appendix, three in the gall-bladder, and three in the stomach. In none was the appendix involved to the exclusion of the gall-bladder or stomach.

The colon bacillus in most cases is to be regarded as a secondary invader because it is found both by cultures and in sections either in decreasing numbers in the tissue from the lumen outward, or is displaced entirely by the streptococci (Cases 128, 184, 191, 205, 210, 211, and 212), and because when injected into animals in pure culture it failed to infect the appendix, except in the case of the strains from Case 203. This accords with the results of Beaussenat cited by Adrian,⁹ who was unable to produce appendicitis by intravenous injection of colon bacilli without injuring the mucous membrane.

In some instances in which mixtures of streptococci and colon bacilli were injected, the former were found in predominating numbers in the appendix as shown both by cultural and by microscopic examinations (Rabbits 798, 846, 847, 859, and 860). The bacillus pyocyaneus, found in the appendix in Case 212, and the diphtheroid bacillus, found

9. Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, 7, p. 407.

TABLE 3
 LESIONS PRODUCED BY INTRAVENOUS INJECTION OF STRAINS ISOLATED FROM CASE 128

Animal	Date of Injection	Bacteria Injected	Amount in c.c. Injected	Date of Autopsy	Lesions in										Remarks	
					Appendix	Stomach and Duodenum		Gall-Bladder	Pan-creas	Joints	Endo-cardium	Myocardium	Muscles	Kidneys		Intestines
						Hemorrhages	Ulcers									
Rabbit 686.....	Aug. 23	Streptococcus from tonsil, first culture	45	Aug. 24	++	+	0	0	0	+	0	0	0	0	0	Small hemorrhage just beneath the mucosa
Rabbit 689.....	Aug. 24	Streptococcus from tonsil, second subculture	45	Aug. 26	+	0	0	0	0	0	0	0	+	0	0	
Rabbit 692.....	Aug. 25	Same as Rabbit 686 after streptococcus kept in NaCl solution for 48 hours	20	Aug. 26	0	0	0	0	0	+	0	0	+	0	0	
Rabbit 693.....	Aug. 25	Same as Rabbit 686 after streptococcus kept in NaCl solution for 48 hours	20	Aug. 26	++	0	0	0	0	+	0	0	0	0	0	Sections of the appendix show hemorrhages in the mucosa, margin of lymph follicles, and under the peritoneal coat Overwhelming infection Overwhelming infection
Rabbit 702.....	Aug. 26	Streptococcus from tonsil, third subculture	50	Aug. 27	0	0	0	0	0	+	0	0	0	0	0	
Rabbit 703.....	Aug. 26	Streptococcus from tonsil, third subculture	75	Aug. 27	+	0	0	0	0	+	0	+	+	0	0	
Rabbit 687.....	Aug. 23	Colon bacillus from pus in appendix	75	Aug. 24	0	0	0	0	0	0	0	0	0	0	0	
Rabbit 704.....	Aug. 26	Colon bacillus from pus in appendix	60	Aug. 27	0	0	0	0	0	0	0	0	0	0	0	
Rabbit 705.....	Aug. 26	Streptococcus and colon bacillus from appendix	15	Aug. 27	++	0	0	0	0	0	0	+	0	0	0	
Rabbit 709.....	Aug. 28	Streptococcus from appendix	60	Aug. 31	++	0	0	0	0	+	0	0	0	0	0	
Rabbit 710.....	Aug. 28	Streptococcus from appendix	60	Aug. 29	++	+	0	0	0	0	+	0	0	0	0	
Rabbit 713.....	Aug. 28	Streptococcus from appendix	60	Aug. 31	++	0	0	0	0	+	0	0	0	0	0	
Rabbit 714.....	Aug. 28	Streptococcus from appendix	60	Aug. 29	++	0	0	0	0	0	0	0	0	0	0	
Rabbit 719.....	Sept. 1	Streptococcus from appendix after 1 animal passage	45	Sept. 3	0	+	+	0	0	+	0	0	0	0	0	
Rabbit 720.....	Sept. 1	Streptococcus from appendix after 1 animal passage	60	Sept. 2	0	++	0	0	0	+	0	0	0	0	0	
Rabbit 724.....	Sept. 3	Streptococcus from appendix after 2 animal passages	4.5	Sept. 5	0	++	+	+	0	++	+	+	+	0	0	
Dog 131	Sept. 4	Streptococcus from appendix after 2 animal passages	75	Sept. 5	0	++	+	0	0	0	0	0	0	0	+	
Rabbit 728.....	Sept. 6	Streptococcus from appendix after 3 animal passages	3	Sept. 8	+	++	+	0	0	+	0	+	+	0	0	
Guinea-pig 1234	Sept. 6	Streptococcus from appendix after 3 animal passages	2	Sept. 9	0	++	+	0	0	+	0	0	0	0	0	

Sections of the appendix show hemorrhages in the mucosa, margin of lymph follicles, and under the peritoneal coat Overwhelming infection Overwhelming infection

TABLE 4
 LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF STRAINS FROM CASE 212

[illegible]

TABLE 5
 LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF STRAINS FROM CASE 184

Rabbit	Date of Injection	Bacteria Injected	Amount In c.c. Injected	Date of Autopsy	Lesions in										Remarks	
					Appen- dix	Stomach and Duodenum		Gall- Bladder	Pan- creas	Joints	Endo- cardium	Myocar- dium	Mus- cles	Kid- neys		Intes- tines
						Hemor- rhages	Ul- cers									
S45	November 1	Streptococcus and colon bacillus from appendix	15	November 5	+++	0	0	0	0	0	0	0	0	0	+	Section of the appendix shows many streptococci and a few colon bacilli in areas of hemorrhage
S46	1	Streptococcus and colon bacillus from appendix	15	2	+++	0	0	0	0	0	0	0	0	0	+	
S47	1	Streptococcus and colon bacillus from appendix	1.5	3	+++	0	0	0	0	0	0	0	0	0	0	
S49	1	Streptococcus and colon bacillus from appendix	30	2	+++++	0	0	0	0	0	0	0	0	0	+	
S87	8	Streptococcus and colon bacillus from peritoneum	45	9	+++	0	0	0	0	0	0	0	0	0	+	Sections of the appendix show many streptococci on areas of hemorrhage and necrosis. Injection made into lumen of appendix
S88	8	Streptococcus and colon bacillus from peritoneum	20	9	+++	0	0	0	0	0	0	0	0	0	0	
S85	2	Streptococcus from peritoneal coat	30	3	+	0	0	0	0	0	0	0	0	0	0	
S51	2	Streptococcus from peritoneal coat	30	4	+	0	0	0	++	0	0	0	0	0	0	
S52	2	Streptococcus from peritoneal coat	15	5	+	0	0	0	0	0	0	0	0	0	0	
S53	2	Streptococcus from peritoneal coat	15	3	++	0	0	0	0	0	+	0	0	0	0	
S54	2	Streptococcus from peritoneal coat	30	3	+++	0	0	0	0	0	+	0	0	0	0	
S55	2	Streptococcus from edematous peritoneal coat	30	4	0	0	0	0	0	0	0	0	0	0	0	
S64	4	Streptococcus from peritoneal coat	60	6	++	0	0	0	0	0	0	0	0	0	0	
S65	4	Streptococcus from peritoneal coat	30	6	++	0	0	0	0	0	0	0	0	0	0	
S66	4	Streptococcus from peritoneal coat	30	6	++	0	0	+	0	0	0	0	0	0	0	
S57	3	Streptococci and colon bacillus from tonsil	15	4	++++	+	0	0	0	0	0	0	0	0	+	Section of the appendix shows many streptococci and a few colon bacilli in areas of hemorrhage
S58	3	Streptococci and colon bacillus from tonsil	15	4	++	+	0	0	0	0	0	0	0	0	+	

859	3	Streptococcus and colon bacillus from tonsil	30	4	++	0	0	0	0	0	0	0	0	0	0	0	0	Sections of the appendix show many streptococci in areas of hemorrhage. A few subcutaneous hemorrhages.
860	3	Streptococcus and colon bacillus from tonsil	30	4	++++	0	0	0	0	0	0	0	0	0	0	0	+	
860	2	Streptococci from tonsil	45	9	++	0	0	0	0	0	0	0	0	0	0	0	0	
877	6	Streptococcus from appendix after one animal passage	60	7	+	++	0	0	0	0	0	0	0	0	0	0	0	
878	6	Streptococcus from appendix after one animal passage	45	8	0	0	0	0	0	0	0	0	0	0	0	0	0	
879	6	Streptococcus from appendix after one animal passage	60	7	+	0	0	0	0	0	0	0	0	0	0	0	+	
880	6	Streptococcus from appendix after one animal passage	4.5	7	+	+	0	0	0	0	0	0	0	0	0	0	0	
883	7	Streptococcus from appendix after one animal passage	45	8	+	+	0	0	0	0	0	0	0	0	0	0	+	
892	4	Streptococcus from appendix after one animal passage	45	5	+++	0	0	0	0	0	0	0	0	0	0	0	++	
893	4	Streptococcus from appendix after one animal passage	30	5	+++	0	0	0	0	0	0	0	0	0	0	0	+	
895	5	Streptococci from tonsil after one animal passage	10	7	+	0	0	0	0	0	0	0	0	0	0	0	0	
899	5	Streptococci from tonsil after one animal passage	10	7	+	0	0	0	0	0	0	0	0	0	0	0	0	
870	5	Streptococci from tonsil after one animal passage	10	7	0	0	0	0	0	0	0	0	0	0	0	0	0	
875	6	Streptococci from appendix after two animal passages	10	8	0	0	0	0	0	0	0	0	0	0	0	0	0	
876	6	Streptococci from appendix after two animal passages	10	7	+	+	0	0	0	0	0	0	0	0	0	0	0	
892	9	Streptococci from appendix after three animal passages	15	11	0	0	0	++	0	0	0	0	0	0	0	0	0	
894	9	Streptococci from appendix after three animal passages	30	10	0	0	0	+	0	0	0	0	0	0	0	0	0	
889	8	Streptococci from peritoneum	60	9	0	0	0	0	0	0	0	0	0	0	0	0	0	
881	7	Streptococci from tonsil...	45	9	0	0	0	0	0	0	0	0	0	0	0	0	0	
882	7	Streptococci from tonsil...	30	8	0	0	0	0	0	0	0	0	0	0	0	0	0	

Forty-eight-hour culture

Cultivated on Loeffler's serum for 4 days
Second tonsil culture, long chains
Second tonsil culture

TABLE 5.—*Continued*
 LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF STRAINS FROM CASE 184

Rabbit	Date of Injection	Bacteria Injected	Amount in c.c. Injected	Date of Autopsy	Lesions in											Remarks
					Appen- dix	Stomach and Duodenum		Gall- Bladder	Pan- creas	Joints	Endo- cardium	Myocar- dium	Mus- cles	Kid- neys	Intes- tines	
						Hemor- rhages	Ul- cers									
891	November 9	Streptococci from tonsil and colon bacilli from appendix	15	November 11	0	0	0	0	0	0	0	0	0	0	0	Streptococci from sec- ond culture
893	9	Streptococci from tonsil and colon bacilli from appendix	30	10	0	0	0	0	0	0	0	0	0	0	0	Marked hemorrhages in both testicles. Many streptococci and a few colon bacilli
901	11	Streptococci from appendix in ascites dextrose broth 10 days	30	12	0	0	0	0	0	+	+	0	0	0	0	
829	8	Streptococci from tonsil after 8 days cultivation	30	9	+	0	0	0	0	0	0	0	0	0	0	
821	8	Streptococcus from appendix after 8 days cultivation	30	9	0	0	0	0	0	0	0	0	0	0	0	
885	11	Colon bacilli from appendix	30	9	0	+	0	0	0	0	0	0	0	0	+	Colon bacilli, pure cul- ture
886	8	Colon bacilli from appendix	30	9	0	+	0	0	0	0	0	0	0	0	+	Colon bacilli, pure cul- ture

in the peritoneal coat in Case 203, showed no special affinity for the appendix.

Three rabbits were injected directly into the appendix with strains shown to have affinity for the appendix when injected intravenously, but appendicitis did not develop.

In five of the cases (Cases 128, 199, 210, 212, and 96) the hematogenous origin is indicated by the clinical history as well as by the elective affinity for the appendix of the strains isolated from the tonsils and the appendix. In four cases (Cases 167, to be reported elsewhere, 184, 191, and 205) no history of an associated infection was apparent, but an unsuspected focus in the tonsils (or teeth) was found to contain, at the time of the attack, streptococci with an elective affinity for the appendices of rabbits. In two of the cases, the assumed primary focus was found four to twenty-five days later to contain streptococci which no longer had such affinity. In the other cases (Cases 190, 203, 211, 182, and 184) there was no history to lead one to suspect a hematogenous origin, but in all but one the appendix contained organisms which showed an affinity for the appendices of rabbits when injected intravenously. In two of these, the suspected foci did not contain streptococci which had an elective affinity for the appendix. In the rest no cultures were made from the tonsils.

GENERAL DISCUSSION

It must not be supposed that the infections of the appendices in the rabbits described in the foregoing are simply a part of wide-spread lesions due to the injections of large doses. The elective affinity for the appendix is shown, in some instances, when very small doses are injected. Not infrequently, the amount of involvement in the appendix is in proportion to the size of the dose. The relatively small number of organisms present in emulsions of small portions of the human appendices when injected intravenously localized in the appendices of the rabbits in two instances, thus showing that the affinity is not due to some peculiarity the organisms acquired in the cultures. It was found that emulsions of the appendix, especially when showing marked lesions and many bacteria, were exceedingly toxic, which made it necessary to separate the bacteria from the cells by centrifugation in order to demonstrate the localization of the bacteria in the appendix.

Very young and old rabbits are less prone to develop lesions in the appendix on intravenous injection than half-grown rabbits, and the lesions that so develop in the latter are more marked.

The statements of Aschoff and Ghon and Namba to the effect that if appendicitis commonly is embolic in origin it must be due to bacteria having elective affinity for the appendix would seem to correspond to the facts. The evidence in favor of the view that a focus of infection in the tonsil [or teeth] is primary and the infection in the appendix is secondary is strong. The reverse, namely, that the bacteria in the tonsil are brought there by the blood stream, hardly needs to be discussed, because the inflammation in the tonsils is most acute for some time previous to the attack of the appendicitis. In no case was there any localization in the tonsils in the animals. In the one case in which the colon bacillus was found in the tonsil, having presumably been carried there by the blood, the tonsil showed no noteworthy inflammation. The further fact that the strains both from the tonsil and appendix which showed an affinity for the appendix were different than the usual streptococcus normally present in the intestinal tract, which did not show such affinity, also speaks in favor of the view that the primary focus is in the tonsils [or teeth].

The mucous membrane of the appendix, either human or animal, often bulged markedly when the peritoneal coat was dissected away. The lumen of the inflamed appendix in the rabbit was almost invariably free from fecal contents, except at the base, containing usually mucus with flakes of partially digested mucous membrane, while the lumen of the appendix of a normal rabbit usually contained feces to the very tip. These observations indicate that the cause of the colicky pain in human appendicitis, especially when out of proportion to the actual lesion, is largely due to spasms of the muscles, caused most likely by a relative lack of oxygen and increased acidity, the result of local infection, as developed by Graham.¹⁰

SUMMARY

The results of the observations and experiments indicate that appendicitis, in the absence of foreign bodies, commonly is a hematogenous infection, secondary to some distant focus; that it develops when, for some reason or other, the organisms in the focus, usually streptococci, have acquired an elective affinity for the appendix and at the same time gain entrance into the circulation.

The results bear out my theory that a focus of infection is to be looked on, not only as the place of entrance of bacteria, but also as

10. Surg. Gynec. Obst., 1914, 19, p. 360.

the place where they may acquire the varying affinities necessary to infect distant organs and tissues.

From the results in the animals, it seems, as emphasized also by Heyde, Aschoff, and others, that appendicitis is a serious disease, not so much on account of the virulence of the infecting micro-organisms as on account of the anatomy of the appendix favoring strangulation and thus the growth especially of facultative and strict anaerobes.

The importance of thorough search for and removal of possible foci of infection from which appendicitis may originate must be emphasized.

Finally, it may be pointed out that the frequent occurrence of appendicitis, at times almost in epidemic form when throat infections are particularly prevalent, now is easily understood.

EXPLANATION OF PLATES 12 TO 16

PLATE 12

Fig. 1.—Appendicitis in rabbit, twenty-four hours after intravenous injection of a streptococcus from a degenerating colloid goiter in man. Note the hemorrhages and edema in the appendix and the absence of lesions in the cecum. Reflected light.

Fig. 2.—Ulcerative appendicitis in rabbit, five days after intravenous injection of streptococci from rheumatism after one animal passage. Note the marked edema and ulceration of the mucous membrane. Reflected light.

Fig. 3.—Hemorrhagic appendicitis in rabbit, twenty-four hours after intravenous injection of streptococci from tonsil in a case of appendicitis in man (Case 184). Note the marked circumscribed hemorrhages and swelling of the mucous membrane in the appendix and their absence in cecum. Transmitted light.

Fig. 4.—Hemorrhagic appendicitis in rabbit, twenty-four hours after intravenous injection of colon bacilli from the appendix in human appendicitis (Case 203). Note the circumscribed hemorrhages in the appendix and their absence in the cecum. Transmitted light.

Fig. 5.—Appendix of rabbit showing spontaneous retention cysts of the mucous glands. Reflected light.

Fig. 6.—Marked hemorrhages in the duodenum in rabbit, which also had marked hemorrhages in the appendix, twenty-four hours after intravenous injection of colon bacilli from human appendicitis (Case 203).

PLATE 13

Fig. 7.—Small intestine of rabbit showing a swollen, hemorrhagic Peyer's patch twenty-four hours after intravenous injection of a streptococcus from the appendix in human appendicitis after one animal passage (Case 184). The rabbit also had marked appendicitis.

Fig. 8.—Hemorrhages in the appendix and duodenum and hemorrhages and ulceration in the stomach in rabbit, forty-eight hours after intravenous injection of a streptococcus from the appendix in human appendicitis, after three animal passages (Case 128).

Fig. 9.—Appendix removed twelve hours after the onset of acute appendicitis in a young man. Note the necrosis and hemorrhages of the lymph follicle and the marked infiltration throughout the wall. $\times 50$.

Fig. 10.—Diplococci in the peritoneal coat of appendix shown in Figure 9.

PLATE 14

Fig. 11.—Appendicitis in rabbit, twenty hours after intravenous injection of streptococci from the appendix in human appendicitis. Note the hemorrhages and necrosis, particularly of the lymph follicle, and the infiltration and sloughing of the mucous membrane. $\times 600$.

Fig. 12.—Streptococci in the submucosa of the appendix shown in Fig. 11. $\times 1200$.

Fig. 13.—Streptococci in the lymph follicle of the appendix shown in Fig. 11. $\times 1200$.

Fig. 14.—Appendicitis in rabbit twenty-four hours after intravenous injection of a mixed culture of streptococci and colon bacilli from the appendix in human appendicitis. Note the marked hemorrhages and necrosis in lymph follicle, infiltration of the submucosa, and ulceration of the mucosa.

Fig. 15.—Streptococci and a few colon bacilli in the appendix shown in Fig. 14. $\times 1200$.

PLATE 15

Fig. 16.—Human gangrenous and fetid appendicitis, following Vincent's angina. Section of the appendix shows marked necrosis and leukocytic infiltration throughout. $\times 45$.

Fig. 17.—Streptococci and fusiform bacilli in the submucosa of the appendix shown in Fig. 16. $\times 1200$.

Fig. 18.—Hemorrhage, necrosis and leukocytic infiltration in appendix in rabbit, twenty-four hours after intravenous injection of a mixed culture of fusiform bacilli and streptococci from the edematous mesentery in human appendicitis. $\times 55$.

Fig. 19.—Streptococci and fusiform bacilli in the appendix shown in Fig. 18. $\times 1200$.

PLATE 16

Fig. 20.—Colon bacilli in peritoneal coat in a case of subacute appendicitis in man. The organisms resembling diplococci are in partial focus. $\times 1200$.

Fig. 21.—Hemorrhages, necrosis, and leukocytic infiltration in the appendix of rabbit, twenty-four hours after intravenous injection of the colon bacillus isolated from the appendix in a case of colon bacillus appendicitis in man (Case 203). $\times 58$.

Fig. 22.—Colon bacilli in peritoneal coat of appendix shown in Fig. 21. The organisms resembling diplococci are bacilli in partial focus.



Fig. 1



Fig. 2

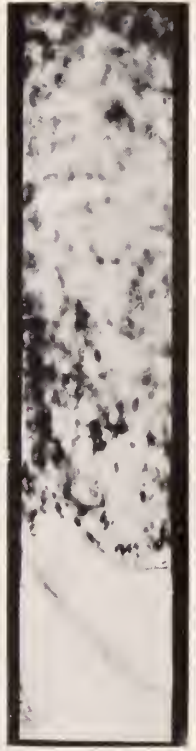


Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7



Fig. 8

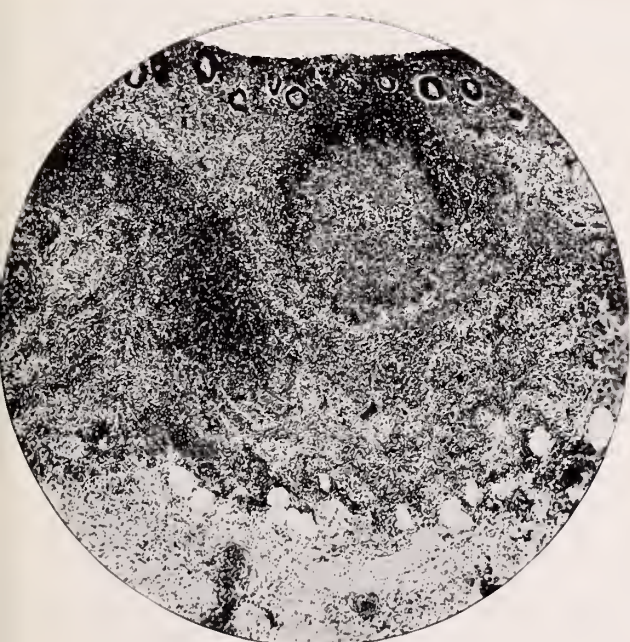


Fig. 9

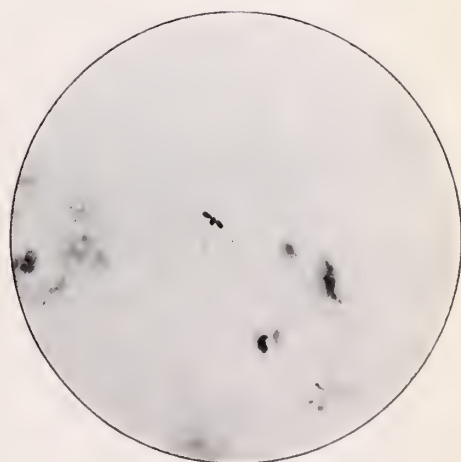


Fig. 10

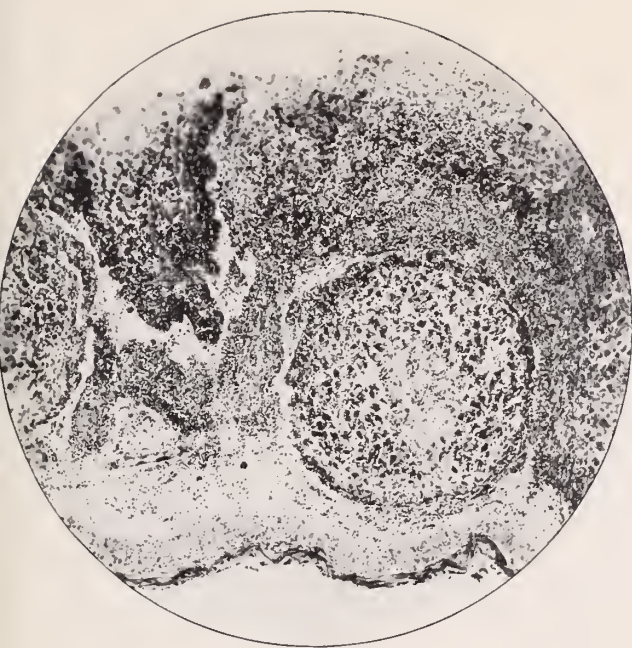


Fig. 11

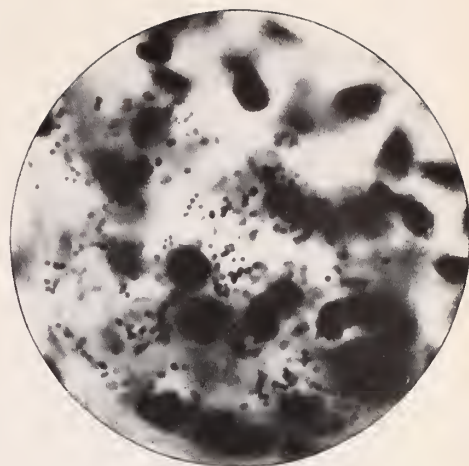


Fig. 12

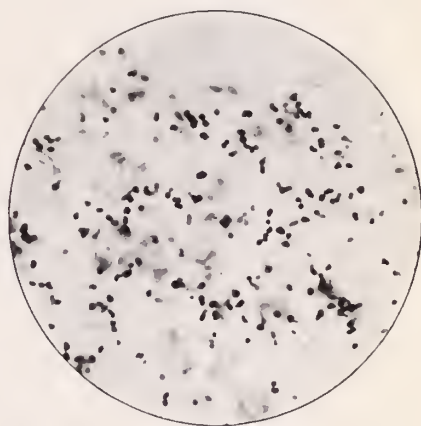


Fig. 13

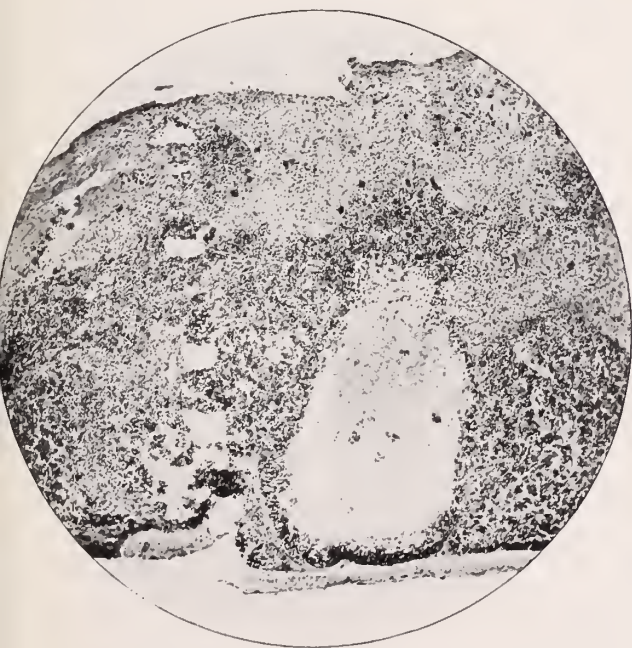


Fig. 14

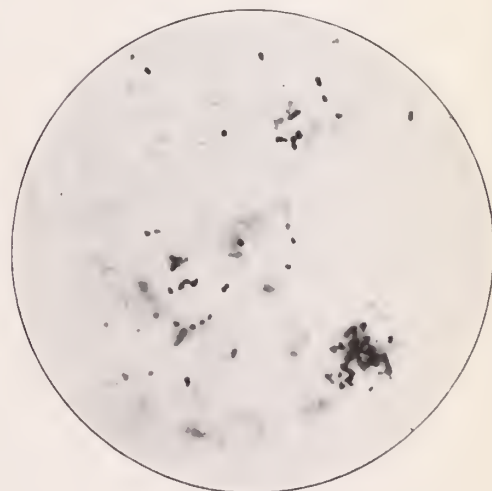


Fig. 15

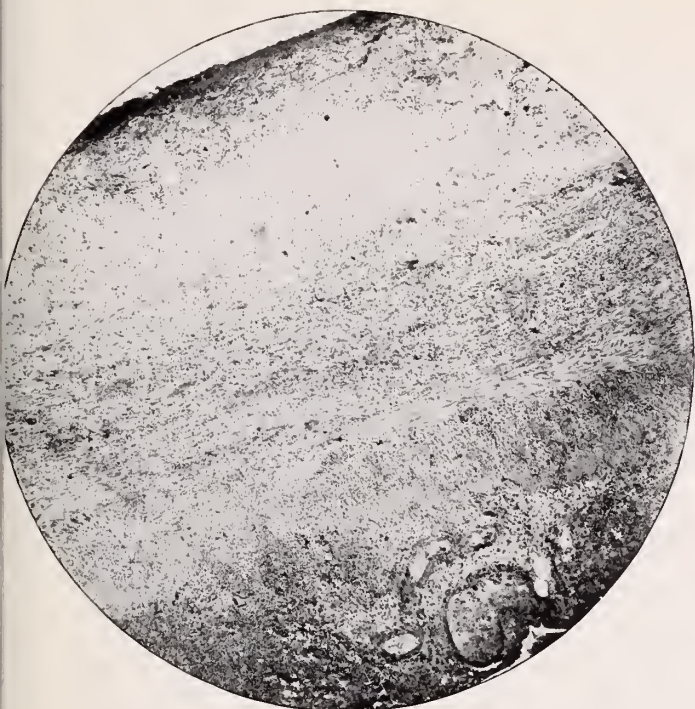


Fig. 16

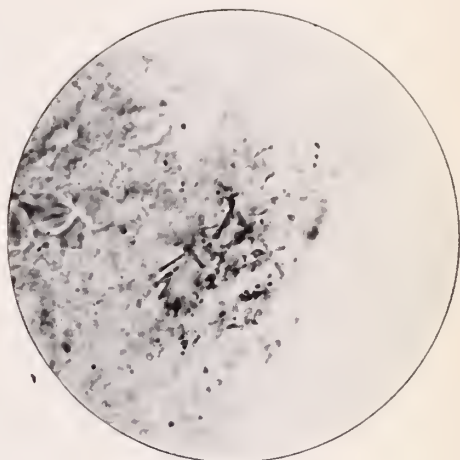


Fig. 17

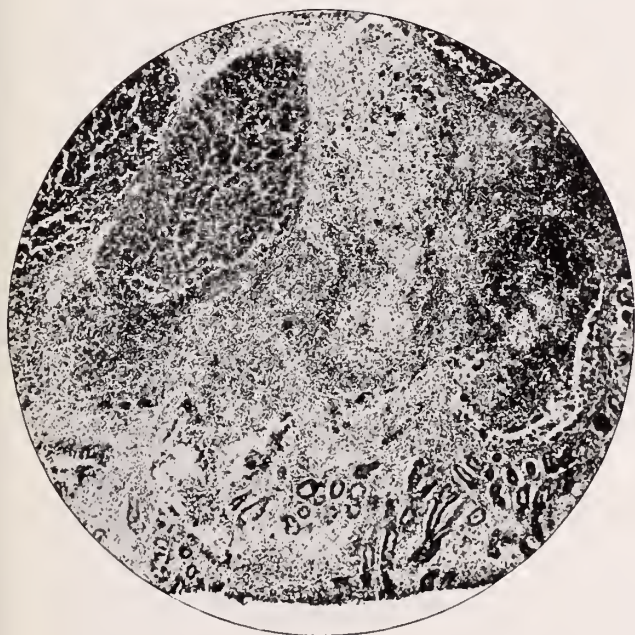


Fig. 18

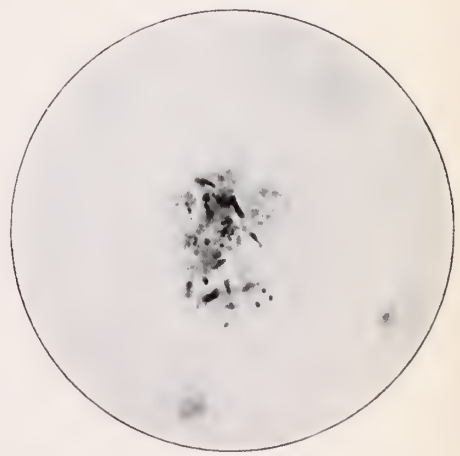


Fig. 19

PLATE 16

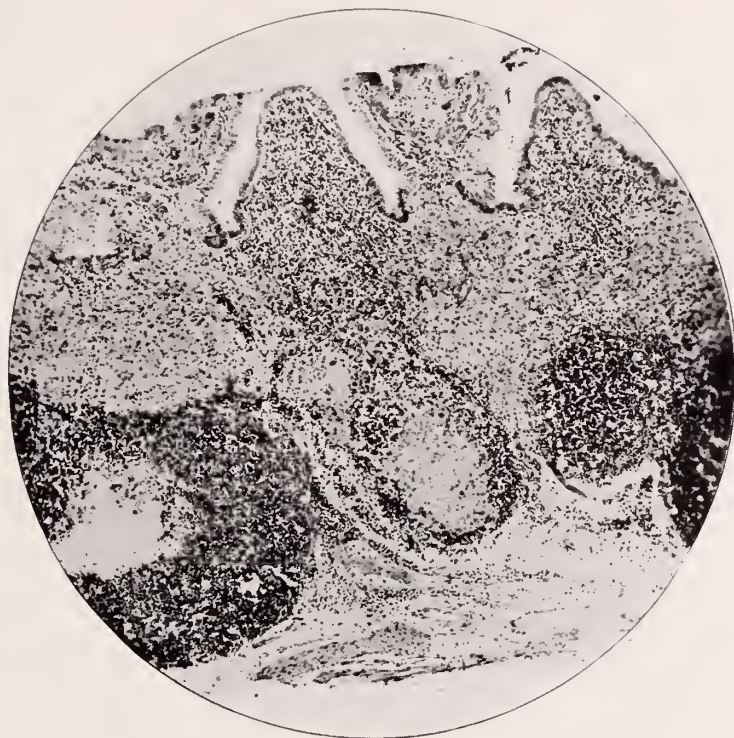


Fig. 21

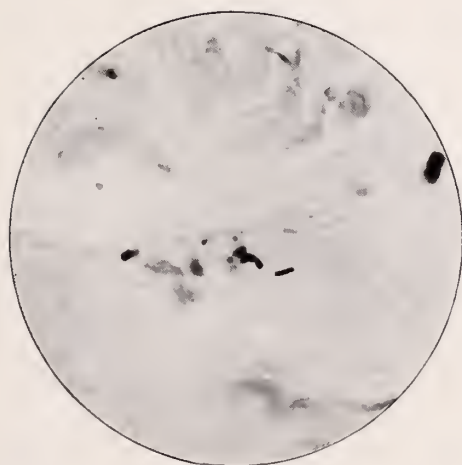


Fig. 20

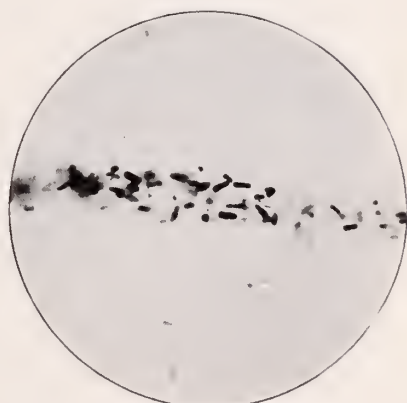


Fig. 22

ON THE SPIROCHETAL INFECTION OF ULCERS IN CHINA *

H. E. EGGERS

(From the Laboratory of Pathology and Bacteriology, Harvard Medical School of China, Shanghai, China)

The occurrence in China of spirochete-infected ulcers of serious clinical importance was first observed by Assmy,¹ who in 1909 called attention to the presence of tropical ulcer in Chungking, a type of ulcer peculiar clinically and characterized by the presence of spirochetes and fusiform bacilli. Later, Logan² reported the frequent occurrence of the disease at Changteh.

The present work was begun to ascertain principally the geographical distribution of the disease. It is based on smears from unselected cases of ulcers of the extremities, contributed for the most part by members of the China Medical Missionary Association, to whom I wish to acknowledge my great indebtedness. I am indebted especially to Dr. H. S. Houghton, chairman of the committee of research. A word of explanation is necessary as to why the material was limited to ulcers of the extremities, in view of the fact that ulcers of the genitalia, of the same general character as tropical ulcer, are known to occur. However probable it may be, it has not been proved that the infection in these cases is similar to that in cases of true tropical ulcer, and it was desirable to keep the subject as free from complications as possible. Second, it was feared that by admitting genital ulcers into the field of investigation a large amount of negative material from venereal infections would be received, which would alter the statistics on relative frequency. In a few instances smears of this character were included among those examined. Their number, however, is too small to appreciably affect the statistical value of the data. The smears, which were sent in without fixation, were fixed in methyl alcohol and examined after staining over night with Giemsa.

Altho the work was primarily intended as a study of tropical ulcer, it was soon found that it would be necessary to broaden it into a study

* Received for publication January 11, 1915.

1. China Med. Jour., 1909, 23, p. 384; Arch. f. Schiff. u. Trop. Hyg., 1909, 13, p. 657.
2. China Med Jour, 1911, 25, p. 224

of the subject of spirochetal infections of ulcers in general in view of the interesting diversity observed in the organisms of that sort. In all, six principal types of spirochetes have been observed.

Type A.—This is a long, tenuous organism, which typically possesses from three to four complete, regular convolutions of considerable amplitude (Figs. 1 and 2). It is on an average about 13μ long, but varies considerably from this figure in both directions. As a rule it takes a bluish stain with Giemsa's solution. Occasional

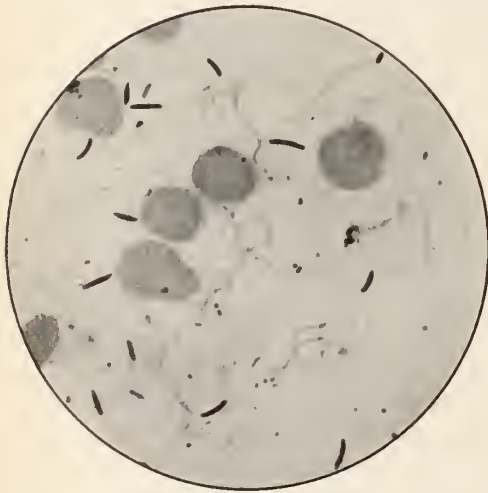


Fig. 1

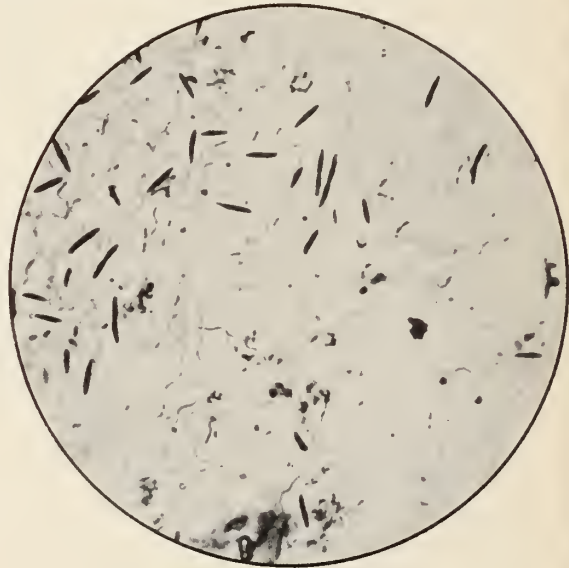


Fig. 2

Fig. 1.—Tropical ulcer, with spirochetes of Type A and fusiform bacilli of Type 1. Giemsa $\times 1000$.

Fig. 2.—Tropical ulcer, with spirochetes of Type A and fusiform bacilli of Type 1. Giemsa $\times 1000$.

smears are found with organisms of this sort stained violet, but this is probably due to irregularity in the stain. Individual organisms sometimes show variations in staining results, as some parts stain deeply while other parts stain scarcely at all. Again, the one organism is at times much thicker at one end than at the other. Scattered among the typical forms described are found spirochetes with perfectly regular convolutions, but much thicker and with tapering ends. These were believed by v. Prowachek,³ who studied the disease in

³ Arb. a. d. k. Gsndhtsamte, 1907, 26, p. 23.

Java, to represent sexual forms. What v. Prowachek considers as resting forms—spirochetes with terminal nodules—are also observed, altho the intermediate stages between these and the normal spirochetes, as described by v. Prowachek, and by Keysselitz and Mayer,⁴ have not been seen by the writer. Occasional organisms show a terminal flagellum. The bending of these spirochetes into irregular loops and volutes and their grouping together in tangled skeins are not infrequent.

There are several variants of this type. The first differs in that the convolutions are smaller, more numerous, and more irregular (Fig. 3). That this does not constitute a specific variation was shown

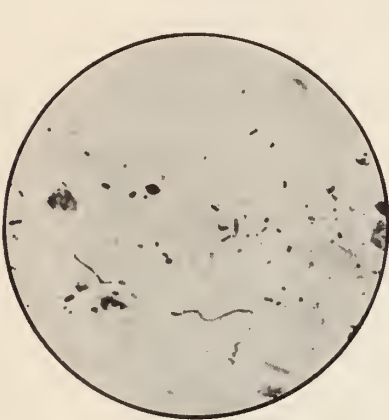


Fig. 3



Fig. 4

Fig. 3.—Irregularly convoluted spirochetes of Type A, with spirochetes of Type F near left margin. Giemsa $\times 1000$.

Fig. 4.—“Straight” organisms of Type A and associated bacilli of Types 1 and 3. Giemsa $\times 1000$.

by an organism of this type which was found linked end to end with one of the regularly convoluted type. The second variant is practically straight, as if a convoluted form had been subjected to tension (Fig. 4). This form occurs as a rule in large numbers in the specimens in which it is present at all. There is little reason to consider this as a separate type as it is found only in association with the convoluted forms and has every appearance of having been derived from them. Another variant, which quite possibly represents a different organism, has been seen only in specimens from Swatow, Kuangtung. It is characterized by great length (17μ average) and by more

4. Arch. f. Schiff u. Trop. Hyg., 1909, 13, p. 137.

numerous convolutions of great regularity and relatively slight amplitude (Fig. 5).

Since the organisms described by v. Prowachek and termed by him *Sp. schaudinni* are commonly regarded as the type of the spirochetes of tropical ulcer, it was of interest to compare the organisms observed in China with these, the more so as the drawings accompanying v. Prowachek's article show an organism considerably less tenuous

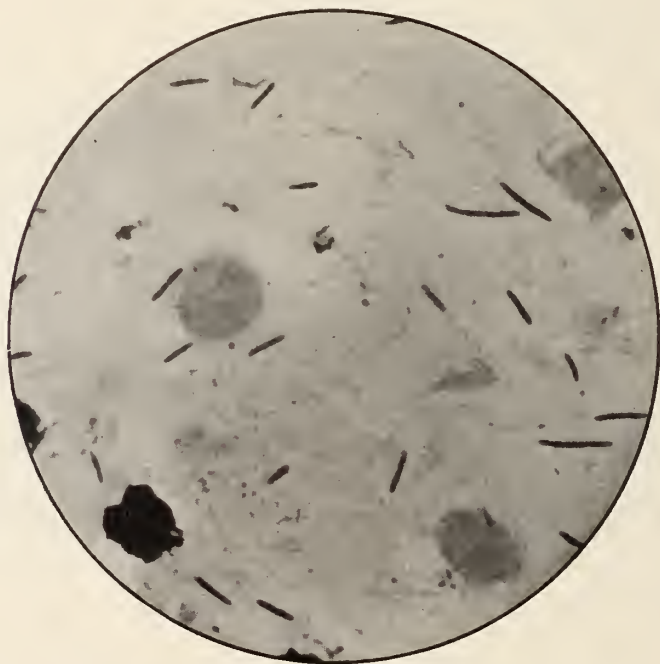


Fig. 5.—Spirochetes of Swatow type and fusiform bacilli of Type 1. Giemsa $\times 1000$.

than the Chinese form. This comparison was made possible by material kindly sent from Java by Dr. H. M. Neeb. The spirochetes in the Javanese specimens apparently differed in no respect from those of the Chinese specimens. The latter also appear to be similar to those described by Keysselitz and Mayer⁴ and Wolbach and Todd.⁵ They differ from the latter, however, as do the Javanese spirochetes, in having the nodular enlargement of the "resting forms"

5. Jour. Med. Research, 1912, 27, p. 27.

at or near the end of the spirochete, while in the forms from Zambesia described by the latter observers the nodules were more nearly central.

Type B.—This organism is a trifle longer and much thicker than Type A. It is irregularly convoluted and stains red or reddish violet with Giemsa. Its ends taper abruptly (Fig. 6).

Type C.—Like Type B, this spirochete stains red or reddish violet. It is a little thicker than Type A and is much shorter, seldom over $7\ \mu$ long. Further, it is characterized almost invariably by perfect regularity of convolutions, which are of small amplitude.

Type D.—This organism, met in only a few cases, is characterized by extreme tenuousness and the possession of only one or one and

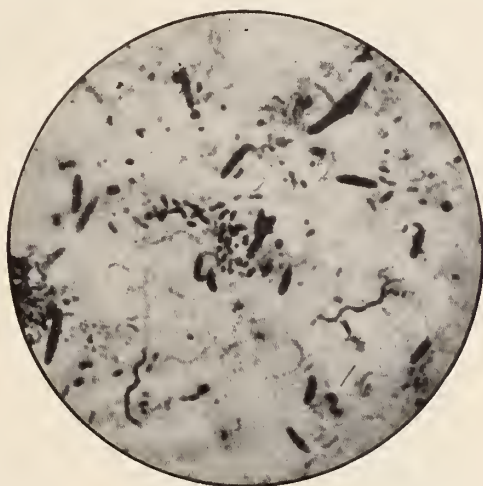


Fig. 6.—Spirochetes of Type A and B (latter in lower half of field) and fusiform bacillus showing central swelling. Giemsa $\times 1000$.

one-half complete convolutions of perfect regularity and great amplitude. It stains bluish violet.

Type E.—Organisms of this type have been met only twice. They are about as long as Type C, very thick, regularly and finely convoluted, with abruptly tapering ends, and they stain an intense blue.

Type F.—This organism is generally of the same tenuousness as Type A, but differs from it in that the former is as a rule irregularly and finely convoluted. It is of about the same length as Type C (Fig. 3). Organisms of this type differ considerably among themselves, and it is quite possible that it includes more than one type.

It is inadvisable, however, to risk undue multiplication of varieties on minor distinctions.

With all spirochetes that occur in large numbers, it is not unusual to find paired forms, to which reference has already been made, linked together end to end by a fine filament. These are the only unquestionable evidences of division that have been observed. Much more doubtful in this connection are the spirochetes with apparently forked ends, which are not infrequent.

Altho there are apparent exceptions, there occur along with the spirochetes certain distinctive bacilli. Following is a description of three of these bacilli:

1. Fusiform bacilli, which stain violet. These may be grouped into two subtypes, altho it is not always possible to differentiate sharply between them: (a) Cytoplasm uniform or finely granular. Sometimes slightly curved; at times forming chains of considerable length. Dimensions of single organism from $6-12\mu$ long, by 1μ in diameter. At times, particularly when forming chains, their ends becoming club-shaped, and they lose their fusiform appearance. Occasionally, they possess a pale-staining, median, transverse zone (Figs. 1, 2 and 5); (b) cytoplasm containing reddish metachromatic granules, usually two in number symmetrically arranged. These bacilli are also frequently curved. They are $3-8\mu$ long and a little less than 1μ in diameter.

2. Fusiform bacilli, much smaller than those of Type 1, with clear, blue-staining cytoplasm and reddish, sharply defined, metachromatic granules (organisms of the Plaut-Vincent type). These, too, differentiate into two fairly distinct subtypes: (a) Very small, very slender, usually with two metachromatic granules symmetrically arranged. Seldom over 4μ in length by 0.5μ , or less, in diameter; (b) longer than the foregoing, usually with more numerous metachromatic granules which frequently, tho not necessarily, are arranged in symmetrical pairs. From $6-9\mu$ long by about 0.5μ in diameter. Also, there are found filaments, sometimes very long, similar to (a) and (b). Usually, these are of greater diameter than the bacilli. While at times they form spirochete-like strands, their differentiation from those organisms never offers real difficulty.

3. Short, plump, violet-staining organisms, with rounded or ovoid ends (Fig. 4). Occasionally these seem to possess a terminal flagel-

lum. Both these and bacilli of Type 1 sometimes show a large, spherical, central swelling (Fig. 6).

This paper is based on the examination of 2,874 specimens, and of these 270, or 9.3 percent, have shown the presence of spirochetes of one or more types. Of these, Type A is the most frequent, occur-



Figure 7



Figure 8

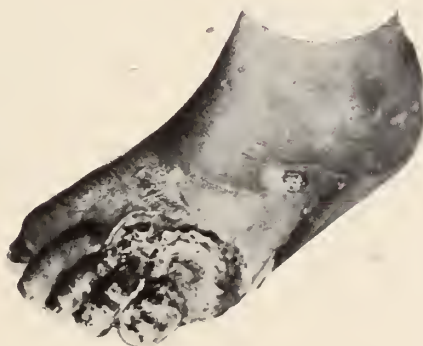


Figure 9



Figure 10

Figs. 7-10.—Lesions of tropical ulcer.

ring in 237 specimens. Type B was found in 36, Type C, D, and E occur infrequently, in 11, 3, and 2 cases, respectively, and Type F is second in frequency only to Type A, which is found in 121 cases.

It must be noted that in only 121 specimens were two or more types of spirochetes found in the same specimen. Not much stress

can be laid on this, for, altho the specimens were examined carefully, usually several times, infrequently one spirochete of a certain type would be found in from 200-400 fields, and when the search is complicated by the presence of overwhelming numbers of one type, as sometimes occurs, associated organisms may easily be overlooked.

As to the numbers in which the spirochetes occur, the greatest variation is shown by Type A, which in some specimens occurs only once in several hundred fields and in others is so numerous as to form a felted mass, with all intermediate stages of frequency. Type B also shows much variation in frequency but is never as abundant as Type A. In some specimens Types C, D, and E occur only in comparatively small numbers. Type F, usually infrequent, is present at times in fairly large numbers, but never presents the striking pictures that Types A and B sometimes show.

Nothing definite could be established concerning the relationship between the fusiform bacilli and the spirochetes. The different types vary in frequency in different specimens, apparently without rule. Occasionally one or more will be absent from a given specimen, and in two smears, both from Hankow, the writer could discover no fusiform bacilli whatever, altho spirochetes of Type A were present in fairly large numbers. On the other hand, fusiform bacilli of all types, and especially of Type 2, have been found, sometimes in great abundance, in a number of specimens in which careful search failed to show the presence of spirochetes. The relationship between the fusiform bacilli and spirochetes is one of symbiosis, probably non-obligative on the part of the fusiform bacilli, possibly mutually so.

At the outset of this work it was decided not to request clinical data in the fear that the amount of extra work involved would result merely in the curtailment of the material received. For this reason little can be said of the clinical significance of the different types of spirochetes, and we assume, as seems probable, that the various morphological types represent different species. Enough data were obtained however, to show that typical tropical ulcer is usually at least associated with large numbers of organisms of Type A. Clinically, these ulcers, as met in China, possess the following principal features (Figs. 7-10): smoothly, steeply margined; edges somewhat infiltrated; the ground covered with grayish, foul-smelling sloughs, which when detached leave readily bleeding granulation tissue. Great pain is an almost constant feature. Logan divides them into two

classes: An acutely fulminating type, running a very rapid course, destroying all tissue down to the bone, and eventually involving the bone itself, and a more chronic type, slowly destroying the soft and bony tissues of the extremity.

In addition, cases are seen in which the infection remains superficial. In the writer's experience these cases, as the preceding, are usually characterized by much pain. The great majority of these infections are single, but multiple ones (auto-inoculation?) sometimes occur. In general, it may be said that the clinical picture of tropical ulcer as met in China is in conformity with that of other regions.⁶

The fact must be emphasized that conditions practically indistinguishable from the clinical standpoint, but without the peculiar



Fig. 11.—Phagedenic ulcer, not associated with spirochetal infection.

bacteriological findings, may occur. Material received from Changteh illustrates this (Fig. 11). It was accompanied by a history typical of the acutely fulminating type of tropical ulcer in every respect except the presence of pain. Smears from this case, however, showed no spirochetes, but a peculiar "bean-pod shaped" organism in almost pure culture. The identification of tropical ulcer from the microscopical viewpoint also offers difficulties. In the course of this work, sufficient data were obtained to make it almost certain that ulcers in which organisms of Type A occurred in small numbers were not as a rule to be regarded as tropical ulcer. As these organisms occur in

6. Some idea of the clinical features as well as the distribution of tropical ulcer may be gathered from the following references:

Regnault (Annam): *Arch. gén. de méd.*, 1904, 2, p. 2268.
 Fontoyont and Gourdran (Madagascar): *Presse méd.*, 1905, 13, p. 35.
 V. Prowachek (Java): *Arb. a. d. k. Gsndhtsamte*, 1907, 26, p. 23.
 Brault (Algiers): *Arch. f. Schiffs- u. Trop.-Hyg.*, 1907, 11, p. 612.
 Keysselsitz and Mayer (German East Africa): *Ibid.*, 1909, 13, p. 137.
 Leboeuf (French Congo): *Bull. Soc. de Path. Exot.*, 1908, 1, p. 339.
 Lenz (German East Africa): *München. med. Wchnschr.*, 1908, 60, p. 2045.
 Stevenel (Zinder): *Bull. Soc. de Path. Exot.*, 1911, 4, p. 180.
 Carr (Persia): *Trans. Soc. Trop. Med.*, 1911-12, 5, p. 206.
 Bruce (Zambesia): *Jour. Trop. Med.*, 1911, 14, p. 1.
 Woldbach and Todd (Zambesia): *Jour. Med. Research*, 1912, 27, p. 27.

all intermediate stages of frequency, 'from once in several hundred fields to sufficient numbers to form felted masses, it is evident that to establish a certain criterion is difficult. The classification adopted in Table 1 was based on the presence in the smear of considerable numbers of spirochetes of Type A in predominating proportion. This appears to correspond in general to the clinical classification. A great deal of the difficulty is probably due to the necessity of identifying these spirochetes by morphology alone. The writer has found in one of a number of chancroid cases and in one of gonorrheal urethritis spirochetes indistinguishable from Type A (Figs. 12 and 13) present

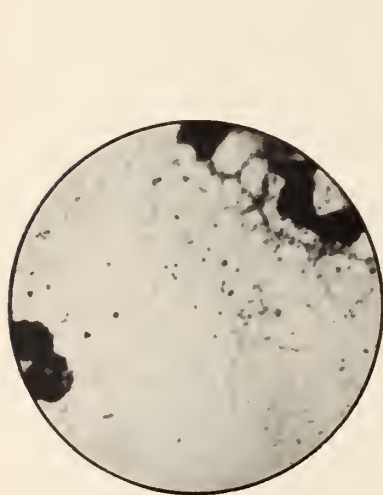


Fig. 12

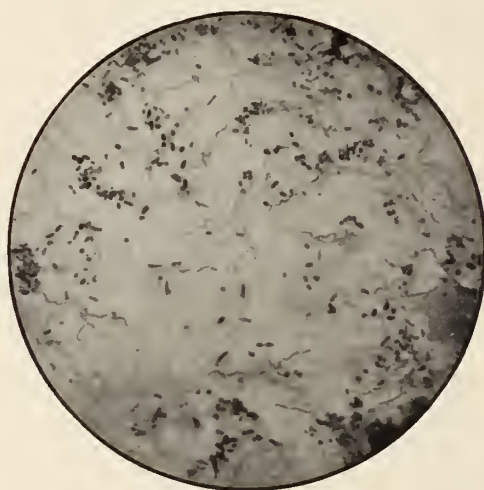


Fig. 13

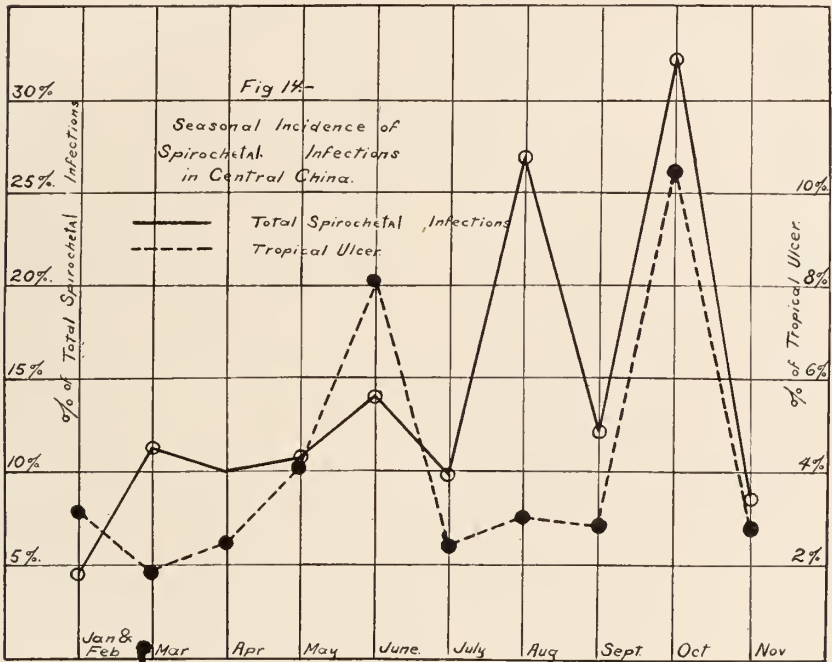
Fig. 12.—Spirochetes occurring in a case of chancroidal infection. Giemsa $\times 1000$.

Fig. 13.—Spirochetes associated with gonorrhoeal urethritis. Methylene blue $\times 1000$.

in considerable numbers, but without apparent influence on the clinical course of these lesions. In both of these specimens there was an absence of fusiform bacilli.

Of the remaining types of spirochetes, Type B was found as a predominating organism in enough cases to possibly warrant the suspicion that it has pathogenic properties. While Type F occurs at times in large numbers, as a rule it is associated with a plentiful and diverse flora and its pathogenicity is less probable. The remaining types of spirochetes occur in too small numbers to make probable any conclusions as to pathogenicity.

In regard to the geographical distribution of these infections, the northern third of China appears to be almost free from them. Out of 282 cases, six showed spirochetes. Of these, five came from barely north of the zone dividing China into three belts of equal latitude, and the sixth case from Peking seems so exceptional as to perhaps warrant the assumption that it was imported. None of these northern cases was of the microscopic type distinctive of tropical ulcer. Central China, using this term to mean the central eight of China's



twenty-four degrees of latitude, shows these infections in the greatest abundance and 221 cases, or 11.3 percent, of a total of 1,950, gave positive results. Seventy-six specimens, or 3.8 percent, could probably be diagnosed as tropical ulcer. All of the 39 centers, from almost all of which a representative lot of material was obtained, showed spirochetel infections. In some, such infections were much more frequent than in others. For instance, Changteh, which leads in frequency, showed 30 cases of spirochetel infection of a total of 66, with included 14 undoubted cases of tropical ulcer. The southern

TABLE 1
GEOGRAPHICAL DISTRIBUTION OF SPIROCHETE-INFECTED ULCERS

Source	Total Specimens	Number Containing Spirochetes	Type A	Tropical Ulcer	Type B	Type C	Type D	Type E	Type F
Kirin.....	18
Sinminfu.....	10
Newchwang.....	13
Total from Manchuria.....	41								
Peking.....	63	1	1	1	1
Paotingfu.....	5
Changli.....	7
Tsangchow.....	11
Shenteh.....	24
Total from Chihli.....	110	1	1	1	1
Taiyuanfu.....	12
Liaochowfu.....	5
Taikuhsien.....	5
Pingling.....	17
Total from Shansi.....	39								
Tehchow.....	10
Taianfu.....	30
Chofoo.....	2
Ichowfu.....	24	4	4
Total from Shantung.....	66	4	4						
Changte.....	5
Weihwei.....	20	1	1
Hwaiking.....	20	2	2
Honanfu.....	5
Total from Honan.....	50	3	3						
Sianfu.....	2
Total from Shensi.....	2								
Paoning.....	11	1	1
Mienchuhsien.....	21	1	1	1
Tungchwan.....	35
Chengtzu.....	41	2	2	1	1	..
Szeliutzing.....	18	5	5	1	1
Chungking.....	134	21	19	5	1	7
Suifu.....	8
Ningyuanfu.....	4
Total from Szechuen.....	272	30	28	7	1	1	9
Fancheng.....	9
Anlu.....	5
Ichang.....	31	1	1
Hankow.....	107	8	8	4	1
Wuchang.....	18	6	6	4	2	3
Total from Hupeh.....	245	17	15	8	2	4
Hwaiyuan.....	19	5	4	1	3	2	5
Luehowfu.....	56	13	13	1	2	..	1	..	9
Wuhu.....	120	10	9	3	4
Anking.....	23	8	7	4	2	5
Total from Anhwei.....	218	36	33	9	7	2	1	..	23

TABLE 1.—(Continued)

Source	Total Specimens	Number Containing Spirochetes	Type A	Tropical Ulcer	Type B	Type C	Type D	Type E	Type F
Hsuehchowfu.....	23	2	2	1
Yangchow.....	49	10	5	2	2	8
Nanking.....	135	12	11	7	2	1	6
Kiangyin.....	17	2	2	1
Wusih.....	39	5	5	1	4
Soochow.....	67	3	1	2	2
Shanghai.....	218	19	16	7	2	8
Total from Kiangsu....	548	53	42	17	6	3	30
Kashing.....	49	4	4	3	3
Huechow.....	103	17	15	3	3	3	7
Hangchow.....	43	8	8	6	1	2
Shaoshing.....	10
Ningpo.....	103	3	3	1	3	3
Taichow.....	36	4	3	2	1	1
Kinhwa.....	5
Total from Chekiang..	349	36	33	15	8	3	16
Dongkau.....	20	1	1	1
Ningteh.....	17
Yengping.....	15	1	1
Foochow.....	38
Futsing.....	10
Yungchun.....	17	1	1
Hweianhsien.....	21	3	3	1	1	2
Siokhe.....	20	5	3	3
Total from Fukien....	158	11	9	2	1	5
Kiukiang.....	57	5	4	1	1	1
Jaocow.....	40	7	6	3	..	1	..	1	3
Nanchang.....	41	2	2	2	1
Total from Kiangsi....	138	14	12	6	2	1	..	1	4
Yochow.....	89	3	2	..	1
Changteh.....	66	30	28	14	4	1	1	..	11
Changsha.....	36	1	1	1
Hengchow.....	11
Yungchow.....	50	5	2	2	1	..	1	..	1
Total from Hunan....	252	39	33	17	6	1	2	..	12
Tengyueh.....	30	1	1	1
Total from Yunnan....	30	1	1	1
Kaying.....	43	3	3	1	1	2
Swatow.....	158	16	14	2	1	10
Canton.....	19
Takking.....	44	1	1	1
Tungkun.....	33	2	2	..	1	2
Yeongkong.....	3
Pakhoi.....	16	2	2
Hoihow.....	8
Kachek.....	2
Total from Kuangtung	326	24	22	3	3	15
Wuchow.....	25	1	1	1
Total from Kuangsi....	25	1	1	1
Unknown.....	5

zone of eight degrees of latitude has contributed 636 specimens from 22 centers, with 43, or 6.8 percent, showing the presence of spirochetes, and 9 specimens, or 1.4 percent, that can with some degree of probability be diagnosed as tropical ulcer. A number of cases of southern infections were contracted during sojourns in Singapore, as there was a large emigration of coolies thither from southeastern



China. A comparison of the incidence of spirochetel infections in central and southern China, as shown by the foregoing data, shows that these infections appear to be relatively more frequent in the former region. The same holds true for tropical ulcer in an even greater degree. Direct comparisons of absolute frequency cannot

be made with the present data, but the statement was made by several contributors from southern China that ulcerative lesions, in general, were rather uncommon there, and the much smaller amount of material sent from the south bears this out. Central China appears to be a richer field than southern China for spirochetel infections, and especially for tropical ulcer.

It is not possible to draw accurate deductions as to the seasonal prevalence of these infections from the material on which this article is based, as very little of it was dated. Approximate conclusions may however be drawn by arranging it according to time of receipt, allowing for the time of transportation from the more remote centers. The results obtained in this way for central China are presented in

TABLE 2
SEASONAL INCIDENCE OF SPIROCHETAL INFECTIONS OF ULCERS IN CENTRAL CHINA

Received	Jan- uary and Febru- ary	March	April	May	June	July	Aug- ust	Sep- tember	Octo- ber	Novem- ber
Total specimens	124	206	290	250	185	165	131	216	171	212
Total specimens containing spirochetes.....	6	23	39	27	26	16	35	26	55	18
Percentage.....	4.8	11.2	10.0	10.8	14.0	9.6	26.7	12.0	32.1	8.5
Type A.....	6	9	26	23	23	15	32	20	55	16
Percentage.....	4.8	4.3	9.0	10.2	12.4	9.1	24.4	10.7	32.1	7.5
Tropical ulcer...	4	4	7	10	15	4	4	6	18	5
Percentage.....	3.2	1.9	2.4	4.0	8.1	2.4	3.0	2.8	10.5	2.8
All other spiro- chetes.....	1	18	28	16	18	6	19	16	44	8
Percentage.....	0.8	8.7	9.6	6.4	9.7	3.6	14.5	7.4	25.8	4.4

Table 2, and graphically in Figure 15. Table 2 shows an increase in the number of spirochetel infections during the hot summer months. With tropical ulcer, on the other hand, the frequency appears to be fairly constant except in the case of material received during the months of June and October, when there is a decided increase. While it is impossible to assert that this is not accidental, the increase may be explained by the fact that rice planting in central China is in May and the harvest in August. This coincides in some measure with this apparent increase in frequency of tropical ulcer, after due allowance has been made for delays between collection of the specimens and arrival for examination. This result of greater incidence

in early and late summer is not in exact accordance with the opinion of a number of observers who are most familiar with the disease, who state that it is greatest during the hot season. Assmu and Kyritz⁷ state that the acutely phagedenic form is particularly frequent during the summer months, and as this is the most recognizable form, it may be the basis for this opinion.

SUMMARY AND CONCLUSIONS

Some six different morphological types of spirochetes have been found in ulcers of the extremities in China. Of these, one is known to be associated with a disease of fairly distinct clinical features. The pathogenic significance of the others is doubtful.

No constant relationship could be shown between the spirochetes and fusiform bacilli, and apparently either may occur independently of the other.

Spirochetes of the morphological type of those associated with tropical ulcer have been found in all degrees of abundance in smears from ulcers. When they are present in small numbers, the lesion apparently presents no distinctive features clinically.

In regard to geographical distribution, the north seems to be almost free from spirochetal infections. They appear to be particularly frequent in central China, and less frequent, tho present, in southern China. The distribution of tropical ulcer is similar.

Some evidence of increased frequency of spirochetal infections was observed during the summer months. Tropical ulcer appears to be most frequent in the early and late summer, tho the data on this point were not sufficiently accurate to warrant indisputable conclusions.

7. Arch. f. Schiffs- u. Trop.-Hyg., 1913, 17, p. 217.

RELATION OF THE NUMBER OF STREPTOCOCCUS LACTICUS TO THE AMOUNT OF ACID FORMED IN MILK AND CREAM *

P. G. HEINEMANN

(From the Bacteriological Laboratory, University of Chicago, Chicago)†

This study was carried on to determine whether or not a relation exists between the number of the streptococcus lacticus and the amount of acid formed in milk and cream. Observations on this question have been occasionally recorded, but systematic investigations have been scarce. Müller¹ found coagulation always took place at 37 C., while sometimes no coagulation occurred at 20 C., even tho a larger amount of acid was formed at 20 C. than at 37 C. Schröder² stated that acid fermentation may take place with greatly varying numbers of bacteria. Luxwolda³ found, after many experiments, that the streptococcus lacticus grows best at 20 C. and that the coagulum formed at this temperature is solid and of pleasant taste. At higher and lower temperatures, competition of other bacteria is more in evidence and so the coagulum is not of desirable quality. Marshall's work⁴ has shown that acid fermentation and coagulation are influenced in a measure by the presence of other bacteria, the activity of which may favor or retard multiplication of the streptococcus lacticus.

It seemed to me desirable to determine the amount of acid formed by the streptococcus lacticus in sterilized milk from day to day and at the same time record the number of bacteria present. It seemed also desirable to compare the results of acid fermentation and coagulation in raw milk in its natural state and of raw milk inoculated with the streptococcus lacticus.

A strain of the streptococcus lacticus with the following characteristics was used: individual cocci were round, gram-positive, and occurred chiefly in pairs, but chains of six to eight members were not uncommon. After numerous transfers, chain formation developed to a degree that chains of sixty to eighty

* Received for publication January 19, 1915.

† This work was aided by a grant from the American Dairy Research Association.

1. Centralbl. f. Bakteriöl., Abt. 2, 1907, 17, p. 345.

2. Original Dissertation, Dresden, 1908 (quoted after Luxwolda).

3. Centralbl. f. Bakteriöl., Abt. 2, 1911, 31, p. 129.

4. Ibid., 1909, 24, p. 22.

members were observed. On dextrose agar a thin veil-like growth developed; on North medium the growth was similar, but more luxuriant. Litmus milk was coagulated within forty-eight hours at 37 C. and litmus decolorized, leaving a pink ring at the surface. After thirty-six transfers in litmus milk coagulation was complete in eighteen hours. Broth was rendered turbid with the accumulation of a diffusible sediment. Gelatin was not liquefied. When inoculated into 250 c.c. sterilized milk, a smooth coagulum was formed in twenty-four hours at 37 C. and a small amount of whey was separated. Nine Erlenmeyer flasks were used for each series. Three series were carried through, two series with milk, from which most of the cream had been removed, and one series with cream. In the first two series each flask was filled with 250 c.c. certified milk and in the last series with 250 c.c. certified cream. Three flasks of each series were sterilized in the autoclave for fifteen minutes under fifteen pounds' pressure. The organism was cultivated for twenty-four hours at 37 C. in tubes containing sterilized milk, and the whole contents of a tube was used for inoculation. The three flasks containing sterilized milk (or cream) and three of the flasks containing raw milk (or cream) were inoculated. Three flasks with raw milk and three with raw cream were allowed to sour spontaneously. The nine flasks of each series were incubated at three different temperatures, 37, 20, and 7 C.

The acidity of the milk and cream was determined before inoculation by titrating 5 c.c. diluted with 45 c.c. distilled water with 0.05 N.NaOH solution, with phenolphthalein as indicator. Plates were prepared both before and after inoculation to determine the number of bacteria present in the raw milk and the number that had resulted from inoculation. Every day thereafter for ten successive days, the amount of acid formed and the number of bacteria present were determined in the same manner. In the cream series, the work was continued for eight days. The culture medium used for plating was dextrose litmus agar of 1 percent acidity. Notes were made on taste, odor, and coagulation.

In Table 1, which gives the results of the experiments, the acidity is expressed in percent of N.NaOH. The numbers given for bacteria should be multiplied by 1,000. A clear view of the results is shown in the plotted curves. In the chart, the acidity is represented by the figures on the side in hundredths percent normal acid. The same figures give the numbers of bacteria in millions. The large dots indicate the approximate time when coagulation was complete. Figures at the bottom indicate the days of incubation.

In sterilized milk with no competition of other bacteria, there was a steady rise in acidity. After ten days the largest amount of acid was formed in milk kept at 20 C., while naturally the smallest amount was formed at 7 C. At 37 C. the milk was coagulated after about thirty-six hours with 540,000,000 bacteria. At room temperature coagulation took place after four days with 350,000,000 bacteria, and at 7 C. there was no coagulation visible after ten days. At 37 C. the number of bacteria decreased after the sixth day, but the acidity

continued to increase. There was no decrease of bacteria in sterilized milk at 20 C. and at 7 C.

In milk not inoculated, the acidity curve ascended constantly for eight days, but the greatest amount of acid was formed at 37 C. Coagulation was complete after two days at 37 C. with 230,000,000

TABLE 1

RELATION OF THE NUMBER OF LACTIC STREPTOCOCCI TO AMOUNT OF ACID PRODUCED UNDER DIFFERENT TEMPERATURE CONDITIONS*

Days	Temperature	Sterilized Milk Inoculated		Uninoculated Raw Milk		Raw Milk Inoculated	
		Acid in N NaOH	Bacteria† on Dextrose Litmus-Agar	Acid in N NaOH	Bacteria† on Dextrose Litmus-Agar	Acid in N NaOH	Bacteria† on Dextrose Litmus-Agar
0.....	37 C.	1.57	0	1.31	0	1.31	0
1.....		2.92	500	6.67	232	7.33	850
2.....		4.05	560	8.45	230	8.18	550
3.....		4.72	585	10.42	130	9.20	190
4.....		4.80	430	10.45	5	9.50	70
5.....		5.10	640	15.10	36	13.90	19
6.....		5.40	705	17.65	29	15.80	17
7.....		5.50	490	18.60	235	16.07	4
8.....		5.80	455	22.30	166	16.02	4
9.....		6.00	375	24.80	243	15.65	1.5
10.....		6.17	310	26.60	260	†	†
0.....	20 C.	1.57	0	1.31	0	1.31	0
1.....		1.57	63	1.23	0	1.63	5
2.....		1.65	255	1.30	14	3.65	46
3.....		1.85	390	3.65	32	6.50	77
4.....		2.00	350	7.60	64	7.90	98
5.....		2.35	930	7.80	106	8.02	120
6.....		3.02	1,300	8.05	221	8.50	75
7.....		4.25	1,335	8.80	267	8.70	57
8.....		5.70	3,150	9.10	105	9.00	39
9.....		7.10	1,980	9.35	80	9.17	21
10.....		7.22	2,600	9.65	54	9.70	17
0.....	7 C.	1.57	0	1.31	0	1.31	0
1.....		1.57	0	1.25	0	1.23	0
2.....		1.57	23	1.17	1	1.16	3
3.....		1.57	39	1.22	1	1.26	14
4.....		1.57	86	1.50	18	1.75	43
5.....		1.65	115	2.62	18	3.15	75
6.....		1.77	157	4.40	112	5.22	26
7.....		2.15	170	6.22	174	6.50	29
8.....		2.50	220	7.35	136	7.45	31
9.....		2.90	355	8.10	76	8.05	32
10.....		3.10	430	8.84	55	8.17	50

* Averages of the two series.

† Contaminated (mold).

† The numbers given for bacteria should be multiplied by 1,000.

bacteria, but the number of bacteria decreased after this and then increased again after the sixth day. At 20 C. coagulation was complete after four days with 64,000,000 bacteria. The number rose to a maximum at seven days after which there was a decline. At 7 C. coagulation was complete after eight days with 136,000,000 bacteria. There was a decrease of numbers on the sixth day. In raw milk

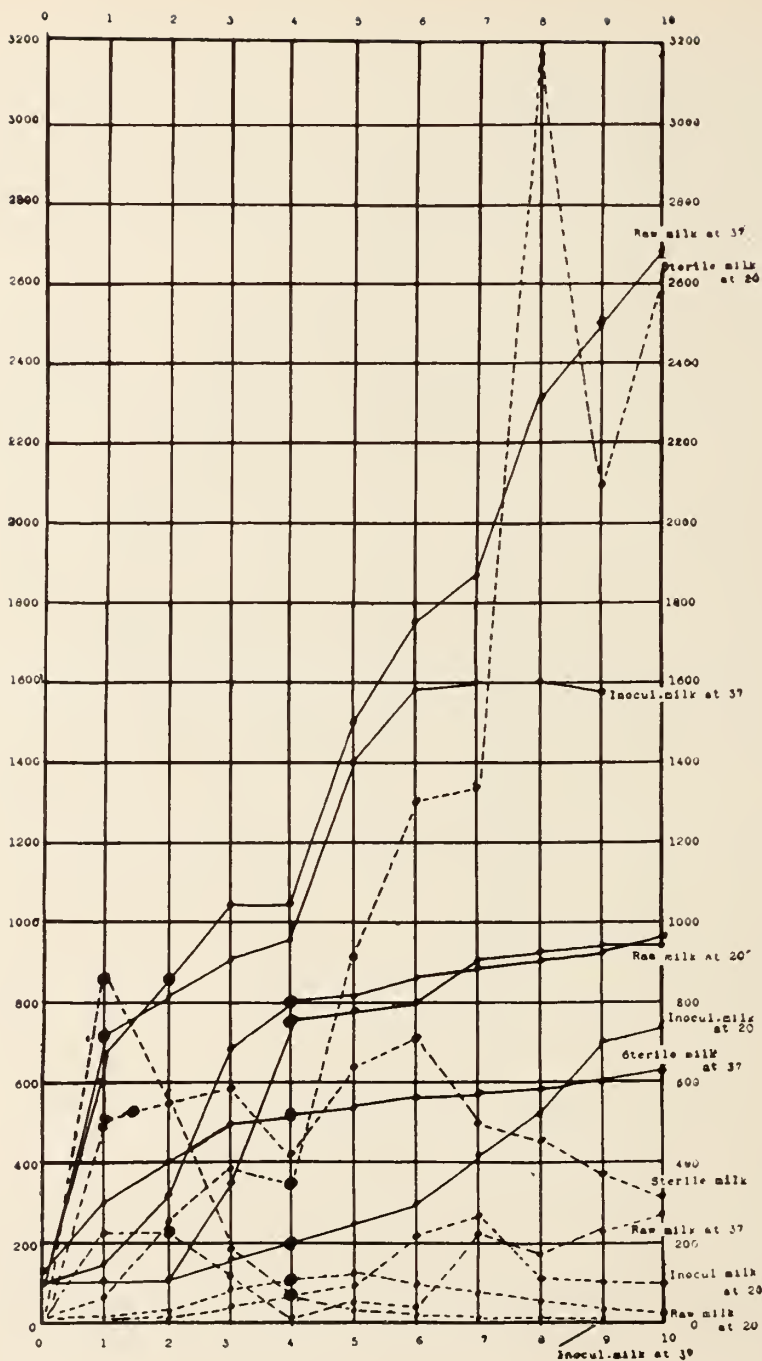


Chart 1.—Amount of acid formed in milk and number of bacteria present. Acid is represented by the solid line, bacteria by the dotted.

inoculated with the streptococcus lacticus, there was a sharp rise of bacterial numbers reaching 850,000,000, after which there was a decided decline. The acidity also rose sharply and continued to rise to the eighth day, after which there was a slight decline. At 20 C. coagulation was reached on the fourth day with 98,000,000 bacteria. There was a slight rise for one day and then a decline. At 7 C. coagulation was complete on the seventh day with 29,000,000 bacteria.

At 37 C. inoculated raw milk was coagulated after twenty-four hours, sterilized milk after thirty-six hours, and raw milk not inoculated after two days. In sterilized milk, coagulation was complete with a smaller number of bacteria and with a smaller amount of acid than in inoculated raw milk, but more time was required for completion of the process. In raw milk not inoculated, the amount of acid present at the time of coagulation was higher than in either of the other milks, but the number of bacteria was relatively low. In both raw milks, enormous amounts of acid were formed at the end of the period of observation. In one case the amount was 26.6 percent (2.39 percent lactic acid), in the other 16 percent (1.44 percent lactic acid).

The amount of acid formed at 20 C. remained below 10 percent (0.9 percent lactic acid) to the end. The number of bacteria in sterilized milk inoculated with the streptococcus lacticus increased constantly with a small interruption from the eighth to the ninth day. In both raw milks a maximum of bacteria was reached, after which there was a decline. At 7 C. the acidity rose consistently with uninoculated milk in the lead, while sterilized milk acidified slowly. The number of bacteria in sterilized milk also rose consistently, while the number in both raw milks reached a maximum which was followed by a decline.

Several points of interest suggest themselves from the results. The enormous amount of acid formed at 37 C. in the raw milk is probably not due to the activity of the streptococcus lacticus alone. Market milk usually contains bacilli of the group of lacto-bacilli, which develop at relatively high temperatures and produce large amounts of acid. These bacteria grow abundantly after the streptococcus lacticus has stopped growing and they form very small colonies. It is probable that in counting colonies large numbers of these have been overlooked. They are difficult to see with small magnification, especially in the presence of other colonies. Examination of plates

with a microscope during the later days of incubation showed that many were present. The assumption that the large quantity of acid formed in raw milk was not produced by the streptococcus lacticus alone is borne out by the fact that in sterilized milk inoculated with this organism the amount of acid remained relatively low.

A second point of interest is the fact that coagulation is not dependent solely on definite numbers of bacteria or a definite amount of acid. It is probable that acid-forming and coagulative enzymes, excreted by bacteria or liberated after disintegration of the cells, play an important rôle in bringing about coagulation. In sterilized milk inoculated with the streptococcus lacticus, the amount of acid increased after the number of bacteria had begun to decrease. This is true chiefly at 37 C., a temperature favorable to enzymic activity. The enzyme, which produces lactic acid from milksugar, is liberated by the large number of bacteria disintegrating and acts even if small numbers of bacteria are present. The large amount of acid produced at 37 C. may therefore be due to the action of enzymes or to the presence of lacto-bacilli, or in all probability to concerted action of both factors. A coagulative enzyme may also be active. The streptococcus lacticus is not known to produce such an enzyme, but, as has been shown by Marshall,⁴ the presence of other bacteria commonly present in milk may aid in the production of acid or of a coagulum. Coagulation may thus be complete with relatively few bacteria present, or with a relatively small quantity of acid.

A third point is the fact that the number of bacteria reaches a maximum and then decreases. The higher the temperature the earlier the maximum is reached. The cause of this may be ascribed to the accumulation of metabolic products, notably acid. Conditions become unfavorable, multiplication stops, and the bacteria disintegrate. This phenomenon is more pronounced in raw milk, where many varieties of bacteria live in competition, than in sterilized, inoculated milk. But even here there is at 37 C. a distinct decrease after six days and at 20 C. after eight days. At 7 C. there is no decrease of numbers for ten days.

The foregoing discussion is based on the average of two series of experiments carried out with the same strain of the streptococcus lacticus, but with two different lots of milk. There is some difference in the number of bacteria and the amount of acid formed in the two series, but the general relation is similar. Generalization would be

unjustified from these results as different strains of the streptococcus lacticus and different kinds of milk used as culture media might lead to different results. However, it may be assumed that the experiments give a fair picture of the phenomena occurring during the process of milk souring. This view is confirmed by the results of the work with cream. The figures are similar to those obtained with milk and warrant the same conclusions to be drawn.

CONCLUSIONS

The amount of acid formed in the souring process of milk or cream is not dependent solely on a definite number of bacteria of the streptococcus lacticus group. Temperature and the presence of other bacteria may influence the result.

In raw milk or cream, or in raw milk or cream inoculated with cultures of the streptococcus lacticus, the number of bacteria increases to a given point and then decreases. At 37 C. the maximum is reached after twenty-four hours and at lower temperature after several days.

Coagulation of milk or cream is not solely dependent on a definite amount of acid or a definite number of bacteria. This absence of definite relation between coagulation, on one hand, and acid and number of bacteria, on the other hand, may be due to the kinds of bacteria present; the kind of acid formed; and the activity of the enzymes produced by bacteria.

At 37 C. extraordinarily high amounts of acid may be produced after several days, due probably to the activity of enzymes produced by the streptococcus lacticus and to the presence of members of the group of lacto-bacilli.

BACTERIEMIA DUE TO BACILLUS DIPHTHERIAE *

H. WINDSOR WADE

(From the Pathological and Bacteriological Laboratories, School of Medicine, Tulane University of Louisiana and the Charity Hospital, New Orleans, Louisiana.)

Clinical diphtheria is a thoroughly studied disease which is characterized by the localization of *B. diphtheriae* on certain of the mucous membranes, usually of the larynx, pharynx, or nasal cavities, from which original foci the infecting agent may spread by continuity or by transplantation. The toxins, evolved by the metabolic activities of the bacteria in the affected tissues, are rapidly absorbed, and it is the presence of these bodies circulating in the blood stream which rapidly gives rise to systemic symptoms.

Certain variation in the site of infection and in pathogenicity of the invading organism should be borne in mind. Not infrequently, cases of what has been termed "concealed diphtheria" occur, in which infection has been established for some time in inaccessible regions, usually the nasal cavity, before extending sufficiently to be recognized.

REVIEW OF LITERATURE

Terrien¹ has reviewed this condition at some length and cites numerous cases in which the throat was not invaded at any time. Altho it is not uncommon for the deeper air passages and the lungs themselves to become involved by the extension of a diphtheric process, these tissues are but very infrequently the primary site of the infection. More or less numerous cases of diphtheric infection of various other tissues occur, either primarily or as a result of transplantation. The list of these is extensive, including the eye, ear, esophagus, stomach, bladder, vagina, rectum, and various parts of the skin surface, and even a meningeal complication has recently been reported. As for the toxicity of various strains of the diphtheria bacillus, there have been reported very wide differences, and some strains, otherwise typical, produce no diphtheria toxin.

While the infection itself is usually purely focal the organisms may enter the blood stream in greater or lesser numbers. The frequency of this invasion and the time and extent of its occurrence are difficult questions. The post mortem bacteriological examination of the blood and viscera has yielded some information. Loeffler is said to have found the organism in the inner organs, and several workers have published the results of studies of such material. These findings seem to be exemplified fairly well by those

* Received for publication January 21, 1915.

1. *Ann. de méd. et chir. inf.*, 1911, 15, p. 5.

2. *Jour. Boston Soc. Med. Sc.*, 1898, 2, p. 92.

of Pearce.² He made cultures from the heart's blood, spleen, liver, and kidney of a large series of fatal cases of uncomplicated diphtheria and of diphtheria complicated with scarlet fever, measles, and bronchopneumonia, and found the diphtheria bacillus in one or more of these sites in a fairly large proportion of cases, either alone or in conjunction with streptococci. The latter type of organism was found alone in a larger percentage than the diphtheria bacillus alone, particularly in the complicated cases. Particularly pertinent to the present discussion is the fact that the diphtheria bacillus was isolated from the heart's blood much less frequently than from the solid organs. In this series, it was recovered from the blood in but six of one hundred and forty cases, or 4.3 percent. In three instances, it was mixed with streptococci, in the others it was recovered in pure culture. In Banhoff's³ series of three hundred and fourteen fatal cases it was recovered from the heart's blood in thirteen instances (4.1 percent), in only three of which cases was it unmixed. In none of these reports is the concentration of the organisms indicated, nor is there any possibility of ascertaining over what period of time the organisms had been present, that is, whether the hematic dispersion was not terminal. Pearce remarks that positive results of tissue cultures occurred most frequently, tho not always, in the gravest cases, known as "septic" diphtheria.

Under the name "diphtheria septique," certain authors designate a very severe, overwhelming type of diphtheric infection, a vivid description of which is given by Baginsky,⁴ who speaks of it as "septicemic general diphtheria." This is not synonymous with diphtheric septicemia (or bacteriemia) for the former appellation depends solely on the clinical manifestations and in no way refers to actual determination of "septic," or other invasion of the blood stream by micro-organisms.

Definite diphtheria bacteriemia characterized by the persistence and multiplication of organisms in the blood stream is evidently very infrequent, and there are but few reported instances in which there is even a strong probability of its occurrence. Hiss and Zinsser⁵ even go so far as to assert that while the organisms may be found in the organs post mortem we have no data which would justify the assumption that a true diphtheria septicemia may occur during life.

Howard⁶ described very carefully an interesting instance of ulcerative endocarditis due to the diphtheria bacillus, in which the point of entry was not determined. Cultures from various organs and sections of tissues, removed eight hours post mortem, showed more or less numerous organisms identified as diphtheria bacilli. The incidence and number of these were held to indicate a bacteriemic invasion secondary to the endocarditis.

3. Ztschr. f. Hyg. u. Infektionskrankh., 1910, 67, p. 349.

4. Quoted by Jacobi: Diseases of Children, 1910, p. 739.

5. Textbook of Bacteriology, 1910, p. 511.

6. Am. Jour. Med. Sc., 1894, 108, p. 651.

Neissen,⁷ having recovered the Klebs-Loeffler bacillus in pure culture from the blood of a 9-year-old boy, published apparently the first case of ante mortem blood stream isolation. This organism was not subjected to pathogenicity tests. Roosen-Runge's⁸ case was probably one of a true septicemia, since the first culture was confirmed by a second positive blood culture and by the post mortem discovery of an ulcerative endocarditis in which the diphtheria organisms were demonstrated. Mahler⁹ secured the organism in two out of three blood cultures in his case, indicating either a considerable scarcity of organisms or an inconstancy of the blood invasion. Ucke's¹⁰ report was based on the discovery of a single colony of an organism morphologically the diphtheria bacillus on one of several tubes inoculated with the patient's blood by a confrère and brought to him for examination. A second blood culture was negative. Diphtheria-like organisms were seen among many staphylococci and streptococci in the pus from a gluteal abscess, but these were not differentiated from skin-inhabiting, diphtheria-like saprophytes. In view of these facts and of the proven absence of toxicity in the organism primarily isolated, the possibility of mistaking a non-pathogenic "diphtheroid" for the diphtheria bacillus must, in this case, be particularly considered. Hesse¹¹ obtained a positive blood culture in a case which developed an infective endocarditis due to the same organism. Leede¹² obtained both the streptococcus and an organism identified as the diphtheria bacillus from the circulating blood of a case in which the bacillary invasion did not persist and only the coccus could be obtained from the heart's blood at autopsy. Morgan,¹³ who has most recently reviewed the literature, records a case in which he isolated an organism corresponding to the diphtheria bacillus from the venous blood thirty-six hours ante mortem.

It is particularly interesting that in the seven cases reported as instances of ante mortem blood invasion of the diphtheria bacillus, the organisms isolated were said to be either entirely non-pathogenic, or of extremely low virulence in the four cases in which animal tests were performed. These cases were those of Roosen-Runge, Mahler, Ucke, and Morgan. Howard's organism, recovered post mortem, was also non-pathogenic.

In recent years, several attempts have been made by cultural work with urine from patients in various stages and with various types of diphtheria and from convalescents, to determine whether or not this disease is essentially a bacteriemia. Nicoll and Wilcox¹⁴ investigated this question in 1913, and reviewed the previous work done. This may be summarized as follows: Conradi and Bierast examined the urine of one hundred and fifty-five patients with fifty-four positive results. None of these cases was followed to study the persistence. But six virulence tests were made, all with positive results. R. Koch examined one hundred and eleven urines from twenty-six patients. In four specimens from two patients, virulent diphtheria organisms were obtained, and in ten specimens from five other patients, diphtheria-like organisms which were either not obtained in pure culture or which were non-virulent were also obtained. Beyer reported results which were almost incredible, since in nineteen cases the specimens examined daily or every other day gave posi-

7. Wien. med. Wchnschr., 1902, 47, p. 2221.

8. München. med. Wchnschr., 1903, 39, p. 1252.

9. Berl. klin. Wchnschr., 1907, 47, p. 1499.

10. Centralbl. f. Bakteriöl. Orig., 1908, 46, p. 292.

11. Deutsch. med. Wchnschr., 1902, 35, p. 1996.

12. Ztschr. f. Hyg. u. Infektionskrankh., 1911, 70, p. 104.

13. Am. Jour. Dis. Child., 1913, 5, p. 317.

14. Ibid., 6, p. 23, Am. Jour. Obst., 1913, 68, p. 167.

tive results in practically all examinations. It may here be added that Freifeld,¹⁵ stimulated by his interest in a case of persistent pseudomembranous infection of the mucous membrane of the bladder caused by a non-toxin-producing strain of the diphtheria bacillus, culturally examined the urine in ten other cases of diphtheria. From four of these he recovered the organism.

Since the results of the earlier workers had proven inconclusive, Nicoll and Wilcox¹⁴ undertook to satisfy themselves as to the appearance of the diphtheria organism in the urine during the course of the disease. Using rather more favorable media than those previously employed, they studied fifty-six specimens from fifty-four patients in varying stages and with various degrees of infection. But two positive cultures were obtained, and these specimens were negative on second examination some days later. They concluded that their two positive findings may have been due to contamination, and that, tho the organisms may very occasionally gain access to the blood and be excreted by the urine, this fact is of theoretic interest only.

Authentic cases of diphtheria-septicemia are undoubtedly very rare, and it has been thought worth while to describe a case of bronchopneumonia which came under the observation of the writer. On account of the presence of bubonic plague in this city during the summer of 1914 and because of the fear that an obscure case of this infection might pass unrecognized, particularly careful admission examinations were made of all patients who presented themselves with a hyperpyrexia not clearly explainable. In many of these cases in which suspiciously enlarged lymph nodes were present, material for examination was obtained by aspiration of the nodes. Frequently a blood culture was also made. As a consequence of this scrutiny, there was seen in consultation with the Admitting Officer of the Charity Hospital a case suffering from fever and prostration, in which was detected a focus of bronchopneumonia and a bilateral tonsillitis of slight extent. Two throat cultures made at the time of admission later proved negative of the diphtheria bacillus, while a blood culture taken at the same time, twenty-two hours before death, showed virulent diphtheria bacilli estimated by count to have been present in a concentration of about 1,600 viable organisms per cubic centimeter of blood.

HISTORY OF CASE

A. H., a colored boy 7 years of age, was brought to the hospital by his parents July 8, 1914, complaining of fever, pain in the left side of the chest, sore throat, and a considerable degree of prostration. The boy told of having had this sore throat for over a week but, according to his mother, he was rather suddenly taken ill three or four days previously, with moderate fever and a cough, the nature of which is not known.

15. Berl. klin. Wchnschr., 1913, 1, p. 1761.

Examination showed the patient to be rather poorly developed and nourished. A general lymphadenopathy was discovered, the glands being symmetrically enlarged, firm, discrete, and not tender. The femoral, inguinal, axillary, cervical, and epitrochlear nodes were easily palpated, but the femoral and inguinal groups seemed especially enlarged. The heart was increased in size and the pulsations were plainly to be seen over the entire left side. The apex beat was in the mid-axillary line. On auscultation a systolic murmur was noted, clearest at the apex. A small area of dullness was made out in the left axilla, over which mucous râles were heard. Throat examination revealed a small, whitish patch on each tonsil, not typical of diphtheria, and not seriously thought to be such. Voice was slightly hoarse but there was no evidence of respiratory obstruction. Respirations were 60 per minute, the temperature 103.5 F., and the pulse 136, full and bounding.

Altho the case was thought not to be bubonic plague, in view of the sudden onset and the degree of prostration out of proportion to the meager physical findings, the possibility of this infection had to be considered. As a part of the strict precautionary measures in vogue at the time, one of the enlarged femoral nodes was aspirated and smears and cultures made from the few drops of material thus obtained. In order to isolate possibly a virulent pneumococcus from the blood stream, a blood culture was also made. The blood was withdrawn by a sterile all-glass syringe with a capacity of 2 c.c. from a vein in the arm after thorough application of iodine to the skin. Three tubes of warm, melted, sterile agar were inoculated and poured into sterile plates. A flask of sterile broth was also inoculated. Two throat cultures were made on Loeffler's serum slants, one from each tonsil. As the smears from the gland fluid showed nothing suggestive of the bacillus pestis, the patient was sent to a ward, in the service of Dr. J. B. Guthrie.

During the night his respirations were jerky and very rapid. At times he moaned and cried in sleep, and changed his position frequently. His temperature continued to rise, until at 4 a. m., it registered 104.6 F. At this time, altho he was very thirsty, he showed difficulty in swallowing and his voice became husky.

The next morning the areas of lung dullness were greater in size and more easily perceived. He now complained of a considerable pain in right elbow. Both throat cultures examined at this time showed only staphylococci and a few streptococci. The cultures from gland fluid showed no evidence of growth of the bacillus pestis. He continued to do badly, moaning and grunting continuously and frequently complained of thirst and drank freely. At noon he became very restless with weak pulse and rapid, jerky, and grunting respirations. At 12:55 he died, twenty-two hours after the cultures were taken. No post mortem examination.

BACTERIOLOGICAL EXAMINATION

The blood agar plates made on admission were examined on the second day after the boy's death. All showed numerous small colonies, both in the depth and on the surface of the medium. Transplants were made and on the following day, because of the suggestive morphology, a full-grown rabbit was injected subcutaneously with a small, washed-down agar culture. Within thirty-six hours the animal died. Examination post mortem showed an edematous inflammatory lesion at the point of inoculation from which the

diphtheria bacillus was grown in pure culture. Other organs showed nothing but congestion. Cultures from the heart's blood, liver, and spleen were negative.

At the time of inoculation of the rabbit, transplants were made into various media, including several tubes of the sugar litmus serum waters of Hiss. At the end of forty-eight hours, these showed acid production with coagulation in dextrose and maltose, and without coagulation in dextrin and levulose. In lactose and galactose there was only slight acid production in seventy-two hours, and in saccharose, mannite and inulin there was no change. This corresponds very closely to the table of reactions given for the diphtheria bacillus by Hiss and Zinsser. Other cultures and the morphology of young organisms grown on Loeffler's blood serum media were all typical of this organism. The morphology on the various media differed widely.

As the final step in the identification of the organism, the influence of specific antitoxic serum on guinea-pigs inoculated with the culture was tested.

On July 19 three pigs were inoculated with a forty-eight-hour broth growth as follows:

Guinea-pig 1.—Given 1 c.c. subcutaneously. The animal was found dead twenty-four hours later. Diphtheria bacilli were recovered from the local subcutaneous lesion only, and cultures from the spleen and liver showed no growth.

Guinea-pig 2.—Given 1,000 units of diphtheria antitoxin intraperitoneally, and five minutes later 1 c.c. of the broth culture given Guinea-pig 1 was administered subcutaneously. This pig is alive one month later.

Guinea-pig 3.—Given 2 c.c. of the toxic culture subcutaneously, and fifteen minutes later 2,000 units of antitoxin intraperitoneally. The animal was very ill for two or three days, after which it recovered completely.

Many writers emphasize the fact that streptococci or staphylococci are also found in a large proportion of cases in which the diphtheria bacillus is found post mortem in the heart's blood or viscera. These accompanying bacteria are assigned an important rôle in the invasion of the blood stream by the diphtheria organism. It was thought desirable to determine whether or not either of these organisms was present in this case, and with this point in mind, two of the plates were studied in detail on the seventh day of their incubation. With the exception of a few large colonies of frank surface contaminators on one plate, all of the surface colonies were rather dense, opaque, and yellowish white. Each colony showed a central heaping up, and about this elevation was coarsely granular and irregularly punctate.

The deep colonies were rather small, lenticulate to round in shape, and were reddish or brownish in color, probably from absorption of blood pigment. They were firm, and often came out of the agar on the wire as a small, solid, yellowish mass. No other type of colony was present.

Sixty transplants were made from two plates. Twenty of these were from surface colonies; three of them being from evident contaminants transplanted to ascertain the type. Forty of these inoculations were made from a single area on one of the plates, and every visible colony in that area was transplanted. On the following day, smears from fifty-seven of the transplants showed the usual intensely gram-positive bacilli, many of which showed distinct polar bodies. The three contaminants, which were transplanted, were found to belong to the *B. proteus* and *B. subtilis* groups. Fifty more smears were then made directly from other colonies on the original plates; on examination all showed diphtheria morphology.

A striking feature of the plates studied was the large number of diphtheria colonies present. Since the amount of blood used in making the plates was known to be 0.2-0.3 c.c. per plate, it was a simple matter to estimate the number of organisms per cubic centimeter of blood. On one plate there were counted 400 and on the second 418 colonies. If we take the amount of blood used as 0.25 c.c. there were roughly 1,600 organisms viable in vitro per cubic centimeter of circulating blood.

In speculating on this heavy invasion of the blood stream so long before death, it was thought that possibly the organism was of low virulence and had been multiplying in the blood stream for a comparatively long period of time, while producing but moderate symptoms. Altho it was evident from the symptoms and post mortem cultures that the experimental animals died from toxic and not "septic" effects of the organisms injected, no real indication had been obtained of the actual amount of toxin produced by the organism. Experiments were therefore carried out to determine the degree of toxin production in vitro.

A thin layer of neutral broth in a large flask was inoculated with the organism and incubated for nine days. This culture was drawn through a Berkefeld filter and diluted, and guinea-pigs were inoculated. Each guinea-pig was given a total volume of 4 c.c. of dilutions of various strengths, under the skin of the abdomen.

Guinea-pig 1 (275 gm.) received 0.04 c.c. of the original toxic broth, and thirty-six hours after the inoculation the animal was found dead; Guinea-pig 2 (268 gm.) received 0.02 c.c. of original toxin, and sixty hours after the inoculation the animal was found dead; Guinea-pig 3 (261 gm.) received

0.01 c.c. of toxin, symptoms for several days, recovered and was alive and well one month after inoculation; Guinea-pig 4 (252 gm) received 0.005 c.c. of toxin, symptoms slight. Alive and well.

It is therefore to be concluded that the minimum lethal dose of toxic broth produced by this strain of diphtheria bacillus is between 0.02 c.c. and 0.01 c.c. or, roughly, 0.015 c.c. for guinea-pigs of about 250 gm. One cubic centimeter would therefore contain 66.66 M. L. D. for guinea-pigs of this weight, which is a fairly high potency for such broth cultures.

DISCUSSION

While it is not a rare development in diphtheria that the surface infection and pseudomembrane should extend along the air passages or should by inhalation be transplanted to deeper levels, it is rare for the primary infection to be of the pulmonary tissues. Indeed, few authors recognize the possibility of this occurrence. That the present case is of this type can only be suggested in view of the rather meager clinical observations and the absence of autopsy. In favor of the pulmonary origin of the infection the principal evidence is negative. There was no complaint of any affection of the nasal chambers, nor was there any sign of coryza or of embarrassed nasal respiration noted. Altho there was a moderate inflammatory condition of the tonsils, no characteristic pseudomembrane was present, and two cultures made from the pharynx on standard serum showed only staphylococci with a few streptococci. The presence of tonsillitis, even of a non-diphtheric nature, might argue the primary lesion to be here, but it seems improbable that the organisms should have completely disappeared when once established. That the negative report on the throat cultures was erroneous can scarcely be maintained. These facts, together with evident lung involvement, led to the clinical diagnosis of bronchopneumonia, altho the physical findings did not indicate an extensive lesion.

The source of the organisms found in the blood culture can scarcely be questioned. The colonies were discrete and well separated and were uniformly distributed through and on the surface of the medium in the three blood agar plates, in only one of which was surface air contamination found. The technic employed at the bedside was as elaborate as is usually used in blood culture work. Therefore, there is no possibility that the organisms found could have come from the skin or other source of contamination.

The nature of the organism has been thoroughly established. The identification work was carried out with particular care on account of our realization of the danger of confusing organisms of the "diphtheroid" group. Morphologically and tinctorially identifiable as the diphtheria bacillus and similarly fatal to laboratory animals, the culture reacts as this organism in sugar serum waters and in proper broth cultures produces a strong toxin which is neutralized by diphtheria antitoxin. This specific protection by diphtheria antitoxin of animals injected with toxin is held by some to be of considerable importance in the identification of the diphtheria bacillus. Nevertheless, Park and Williams,¹⁶ who emphasize this point, also discuss organisms, like the one from Morgan's case, which are morphologically and culturally similar to the diphtheria bacillus, but which are not toxin producers. They consider these as strain variants, possibly through attenuation.

In no case of so-called diphtheria bacteriemia in the literature was the organism proven typically pathogenic or to produce specific diphtheria toxin, a fact which makes the present case more unusual.

The condition of extensive bacteriemia and concurrent toxemia in the case described was it is believed responsible for the death of the patient, while the pulmonary lesions were yet of but moderate extent. That the bacteriemia was a secondary manifestation of the infection is undoubted, but that it cannot be considered terminal or agonal, comparable to the majority of Pearce's cases, for instance, is maintained on account of the length of time before death at which the culture was taken and the great number of organisms present in the blood at that time. Furthermore, the condition of the patient when admitted, while serious, did not suggest the rapid termination that ensued.

The frequency of a temporary blood invasion, or metastasis, in diphtheria is apparently not very low. In cases of least severity, or in those in which controlling doses of antitoxin are given early, the localized nature of the infection is probably maintained throughout. When, however, treatment is begun very late in the infection, when the patient's resistance is low, in cases of greatest severity and particularly those in which the organisms tend to multiply rapidly rather than to produce especially potent toxin, and finally in those in which local secondary invasion of the streptococcus, staphylococcus,

16. *Pathogenic Bacteria and Protozoa*, 1910, p. 205.

or pneumococcus aids in the dissemination of the organisms, there is a greater probability of encountering a systemic infection. In such cases, the presence of a greater or less number of the bacteria in the organs may be expected as a result of accidental transportation and prompt removal of bacteria from the blood stream by these organs.

In a condition such as that in the case described, in which there was no medication and in which the lesion was apparently pulmonary, the general invasion is not surprising. Here the vascularity and permeability of the affected tissue would play a considerable part in the inauguration of the blood infection on account of more or less constant invasion of the capillaries of the congested lung tissue by the bacteria. Possibly, as Howard suggested in his case, the constant addition of many bacteria to the blood caused the ultimate exhaustion of the inhibiting influences, with subsequent multiplication of the organisms in the circulation.

The superiority of the *intra vitam* blood culture over other methods of studying the bacteriemia of diphtheria is here exemplified. Post mortem studies, however carefully carried out, are inconclusive. Not only are the cases studied of the less common, fatal type, but, in case of positive cultures from the blood, the length of time of the blood infection and the concentration of the organism is entirely unsettled. Other factors vitiating the results of this study are terminal dissemination and post mortem invasion and multiplication. Such findings, therefore, indicate the frequency of ultimate invasion of the blood and deeper tissues in fatal cases, but have little bearing on the ante mortem condition of the average case of diphtheria during the course of the disease. The frequency of negative results, particularly of the heart's blood cultures, in the fatal cases seems much more significant indicating the relative infrequency of general blood infection.

As may be expected when so many determining factors, such as technic, cases and their environment, stages of disease and standards of identification are concerned, the results of urine examinations vary widely in the hands of different observers. These range from the more credible negative findings of Nicoll and Wilcox to the nearly all positive results of Beyer. The apparent value of any of these results seems greatly diminished when one considers that the irregular and inconstant percolation of bacteria through the kidneys and their detection in the urine is depended on to indicate the frequency of diphtheria bacteriemia. It is here necessary but to call attention to the urine culture findings in typhoid fever, a disease essentially a bac-

teriemia early in its course, and in which as high as 95 percent of positive blood cultures have been reported. According to different authorities, the typhoid bacilli are not apt to be found in the urine until the second or third week of the fever, and after that they may be found in 17-25 percent of cases. This comparison also appealed to Freifeld,¹⁵ who furthermore called attention to the work of Stufina and Dietman,¹⁷ who cultivated the vibrio of Asiatic cholera from the urine in 5-6 percent of a series of cholera. It would seem evident, therefore, that no accurate deduction can be drawn as to the occurrence of bacteriemia in an infection from a study of the bacteriology of the urine.

CONCLUSIONS

It is evident from the number of hours ante mortem at which the blood culture was taken and from the great numbers of the bacteria present, that the case presented is one of true bacteriemia due to *B. diphtheriae*. From the clinical findings it is concluded that the bacteriemia was secondary to a diphtheritic bronchopneumonia, which was probably primary. The organism isolated is a strain of the diphtheria bacillus of fairly high toxicity and is the only typically toxic strain known to have been isolated from a similar condition.

In the consideration of diphtheria bacteriemia there should be drawn a sharp distinction between the condition of metastasis, or accidental blood infection or contamination, which might occur in any severe local infection and which apparently not infrequently does occur in diphtheria, and the condition of true bacteriemia, which is of much more serious import and which in diphtheria is apparently extremely rare.

In the determination of the incidence of systemic infection by diphtheria bacillus, the blood culture is the logical procedure since by it alone can one secure direct evidence as to its frequency of occurrence in all types of cases, the numbers of viable organisms present, and, in fatal cases, the length of time before death that the blood dispersion was present.

Urine examinations alone cannot be expected to give any reliable indication of the occurrence of a bacteriemia in diphtheria, not only on account of technical difficulties, but because of the great uncertainty of the appearance of the bacteria in the urine, as indicated by the study of this excretion in typhoid fever, a condition known to be bacteriemic in nature.

17. Quoted by Freifeld: *Berl. klin. Wchnschr.*, 1913, 1, p. 1761.

COMPLEMENT FIXATION IN THE DIAGNOSIS OF GONOCOCCAL INFECTIONS *

ERNEST E. IRONS AND H. K. NICOLL

(From the Memorial Institute for Infectious Diseases and the Presbyterian Hospital, Chicago)

The reaction of complement fixation in sera of persons infected with gonococcus, with the use of preparations of gonococci as antigen, has been developed so that at present it offers valuable assistance in diagnosis. The methods and results in large series of cases have already been reported by a number of workers¹ and the limitations of the reaction fairly well defined. It is generally agreed that strongly positive reactions, usually denoted as +++ or ++, indicate the presence of gonococcal infection. It often happens that sera from suspected cases give reactions less pronounced, and the question arises as to how these slight, or "weak," reactions should be interpreted. In gonococcal infections in which the lesions may be superficial or limited in extent, we should anticipate relatively slight change in antibodies in the blood, and it frequently happens that in vaginitis or urethritis negative, or only slightly positive, complement fixation is obtained.

The following observations suggest the necessity of care in the interpretations of slight, or "weak," reactions and the importance of recognizing the possibility of rather rapid fluctuations in the degree of reaction within the short periods of time.

METHODS

In making the complement fixation tests an antihuman rabbit hemolytic system was employed. This was in daily use in routine Wassermann tests and so the amboceptor, complement, and corpuscle suspension were accurately titrated. Polyvalent gonococcal antigens prepared by one of us were used in conjunction with a polyvalent antigen prepared by one of the leading pharmaceutical houses. Both antigens gave consistent results, but, as our own polyvalent antigens

* Received for publication January 22, 1915.

1. Schwarz and McNeil: *Am. Jour. Med. Sc.*, 1911, 143, p. 693; *Ibid.*, 1912, 144, pp. 369, 815.

O'Neil: *Boston Med. and Surg. Jour.*, 1912, 167, p. 464.

M'Donagh and Klein: *Jour. Path. and Bacteriol.*, 1913, 17, p. 559.

Gardner and Clowes: *New York Med. Jour.*, 1912, 96, p. 734.

Smith: *Am. Jour. Dis. Child.*, 1913, 5, p. 313; *Ibid.*, 1914, 7, p. 230.

Kolmer and Brown: *Jour. Infect. Dis.*, 1914, 15, p. 6.

were not always available, we have employed commercial antigen in most of the later work. The anticomplementary dose of each antigen was determined, and one-fourth to one-third of this was used in tests as a maximum antigenic dose.

The usual precautions were taken with the sera to avoid changes due to laking of the blood and exposure to heat and infection, and all were inactivated before use. Positive and negative controls were maintained; the positive sera coming from clinically typical gonococcal infections in which the diagnosis had been confirmed by microscopic or cultural examinations.

In judging the results of the tests, careful attention was paid to the quantitative feature, and an effort was made to rule out slight degrees of inhibition due to possible anticomplementary elements in the sera. Doubtful or weakly positive tests were repeated, often several times, and closely observed. In this discussion we have arbitrarily designated the degrees of reaction as follows: Complete inhibition, + + +, strong; almost complete inhibition, + +, moderate; less than 50 per cent inhibition, +, weak; slight inhibition, almost complete hemolysis, \pm , doubtful; complete hemolysis, —, negative.

METASTATIC GONOCOCCAL INFECTIONS

In the course of studies on infections by various microorganisms giving rise to metastatic lesions, such as arthritis, complement fixation tests have been made in conjunction with careful clinical and bacteriological examinations. Of fourteen cases of metastatic gonococcal infection, chiefly arthritis, iritis, or sepsis, in which the gonococcus was isolated in culture, strongly positive reactions were obtained in ten, moderately positive in three, and negative in one. The large proportion of positive tests may be due partly to the fact that several of these cases were subjected to repeated tests for several weeks or months.

In a second group of twenty-one cases, clinically undoubted gonococcal infection, mostly arthritis, but without cultural confirmation, thirteen gave strongly positive, six moderately positive, and two negative reactions.

Several hundred tests on other cases in which the diagnosis for one reason or another was not clean cut, or in which the result of the fixation test helped to determine it, were made, but these are of course not directly available in a discussion of the reliability of the test itself, tho they have a confirmatory value.

In a group of twenty-one carefully studied adults who had entirely negative histories and who showed no evidence of gonococcal infection, negative reactions were obtained in nineteen, and "weak" positive in two.

We have met with a few other instances where, in sera from persons in whom we were convinced there was no gonococcal infection, slight fixation occurred with gonococcal antigen in otherwise completely controlled tests. These results were usually obtained when close readings of the tests were attempted. Such close readings are probably safe in an individual case in which variations in degree of reaction from positive to negative or negative to positive are being followed from week to week, but where the diagnosis is to be based to a considerable extent on the reaction we agree with others that a slightly positive reaction should be interpreted with great caution and given the same weight as a slightly positive Wassermann reaction. The weak reaction may represent a beginning reaction which later will become positive, or, on the other hand, may be followed by entirely negative subsequent tests. Group reactions have been suggested as accounting for some of these instances of weak reactions. Another source of error is that arising from very slight degrees of hemolysis in the blood after removal from the patient, even when care is taken to avoid injury of the erythrocytes. Thus, blood from a patient in whom there was no evidence of gonococcal infection was placed in two similar tubes. After clotting, the serum removed from the first tube was entirely clear and gave a negative test; the serum from the second tube was very faintly tinged with hemoglobin, and a weakly positive reaction was obtained. An error such as this may occur, as is well recognized in other complement fixation tests, wherever large series of tests are made, unless the possible anticomplementary effect of slight degrees of hemolysis is considered. When "weak" reactions have been obtained in cases for diagnosis we have usually insisted on a second or a third specimen of blood.

On the other hand, we have not met with a moderate or strong positive reaction in a patient in whom gonococcal infection could be excluded.

GONOCOCCAL VAGINITIS

A series of cases of gonococcal vaginitis in an institution devoted to the treatment of venereal disease in children was studied culturally, and the gonococcus was isolated from twelve cases. Complement

fixation tests were positive in seven, and negative in five cases. Of five other cases from which the gonococcus was not isolated, a positive reaction was obtained in four. The duration of the disease in this series varied from two or three to many months.

A second series of cases, studied in the Durand Hospital of the Memorial Institute for Infectious Diseases, was for the most part from patients admitted for scarlet fever or diphtheria, in whom the vaginitis was discovered by routine examination on admission to the hospital and in many of whom there were no means of knowing the previous duration of the disease. In this series, the diagnosis was determined by clinical appearances and careful microscopical examination of smears, with positive cultures in a number of cases, tho not in all. We believe, however, that non-gonococcal vaginitis was not included among these cases. Of seventeen such cases, five gave clearly positive complement fixation, ten negative reactions, and two weak, or doubtful. In one other case, with purulent discharge in which no gonococci could be found after prolonged examination, a strong positive reaction was obtained. In two other cases of vaginitis, one of which was due to a diphtheroid organism and the other of which showed only colon bacillus, negative reactions were obtained.

The smaller proportion of positive reactions (five out of seventeen) in this second series would be increased if the two weakly positive reactions were included. We believe, however, that for diagnostic purposes at least, slight, or "weak," reactions should be interpreted with great caution and that a positive diagnosis should not be based on a "weak" reaction, altho such a reaction may be in some degree confirmatory when taken with other clinical features.

One reason for the smaller proportion of positive reactions in the second series than in the first is to be found in the character of the material. The patients in the first group were in a hospital for treatment of vaginitis, and the severity and duration of the symptoms and presumably, therefore, the deeper involvement of tissues played a part in determining their presence in such an institution. The patients in the second group came to the hospital for other infections, and the vaginitis, discovered only in routine examination, in a number of instances had excited no comment. It is in the detection of this class of cases, however, that hospitals need most assistance.

Of sixteen patients, suffering from diphtheria or scarlet fever without evidence of vaginitis, who were selected as controls, thirteen gave

negative reactions, and three "weak" reactions. Of these three, two were suffering from diphtheria, one of them a boy of twelve in whom gonococcal infection could be excluded.

In sera from patients suffering from scarlet fever, we repeatedly noted an early delay in hemolysis in both the tubes of the Wassermann and gonococcus fixation tests.

As a means of detection of gonococcal vaginitis in the routine examination of children, the complement fixation test has a limited value, for positive reactions could not be expected in cases of early infection, and probably not in cases of longer standing in which deeper involvement has not occurred. In one suspected case, however, the occurrence of a strongly positive reaction led to the repetition of cultures, from which we finally were able to obtain the gonococcus.

From our experience, we should not be willing to accept a negative reaction as conclusive evidence of the absence of gonococcal vaginitis.

VARIATION IN THE DEGREE OF COMPLEMENT FIXATION

The tendency of gonococcal infection to relapse and to develop metastatic complications, together with the failure of one attack to protect against subsequent infection, is well recognized. Recurrences of arthritis and iritis at irregular intervals are often seen. In some of the chronic types of arthritis the exacerbations of joint symptoms may appear occasionally at periods with a more regular interval, so that the patient is able to predict with a fair degree of accuracy the onset of pain and effusion in the joints. Thus, a man who had suffered for five years from a multiple gonococcal arthritis involving both knees and to a less extent other joints had exacerbations of the effusion in the knees at intervals of approximately ten to twelve days. In another man a gonococcal synovitis of the wrist recurred every seven days. In this instance, the interval suggested some relation of the recurrences to the weekly routine of work.

Observations such as these suggest rapid fluctuations of immunity, which, if demonstrable by exact methods, should be taken into account if such tests are to be employed in diagnosis.

A man, 20 years of age, following exposure on January 16 and second exposure January 23, complained of dysuria January 24 and purulent urethral discharge January 25. Previous infection was denied, and from a consideration of other evidence we believe this statement correct. On February 1 there was unusually pronounced edema, with profuse purulent discharge, from which the gonococcus was obtained in cultures. Throughout the period of observa-

tion, treatment was confined to local cleansing, hexamethylenamin by mouth, and later local irritations with a silver albuminate. The course was that of an ordinary gonorrhea in an otherwise normal man without demonstrable complications aside from extension to the prostate.

TABLE 1
COMPLEMENT FIXATION TESTS OBTAINED IN CASE JUST DESCRIBED

Date	Complement Fixation Gonococcus	Wassermann	Symptoms
Feb. 1...	++	—	Profuse discharge, marked edema
Feb. 8...	+ weak	—	Discharge decreasing
Feb. 15...	+ weak	—	
Feb. 22...	—	—	Very slight discharge
March 1...	+ weak	—	Prostate enlarged and tender
March 6...	+ very weak	—	Discharge increased for four days
March 15...	+ weak	—	Discharge decreasing
March 23...	+++	—	Discharge slight
March 29...	+++	—	
April 5...	+++	—	Discharge very slight. Gonococci present.
April 12...	++	—	No discharge noted for two days
April 19...	++	—	
April 26...	—	—	No discharge
May 3...	++	—	No discharge noted by patient
May 10...	+++	—	Gonococci present. Prostate slightly enlarged.
May 17...	++	—	

The early appearance of a positive reaction and the fluctuation of the reaction during the course of the disease are of interest.

A woman of 46 was the subject of an arthritis of both knees in which the effusion recurred regularly every ten days, so that the clinical picture closely resembled that known as "intermittent hydrarthrosis." The first arthritis involving the knees and one elbow occurred nine years previously, following a pelvic infection of known gonococcal origin. At present, there is no evidence of active pelvic infection, but fixation and displacement of pelvic organs are demonstrable on bimanual examination. After three months the arthritis became less severe and finally ceased for five years. The arthritis of the knees then returned with recurrences of pain, followed by distention of the joints without any other marked constitutional disturbance, every eleventh day for eight months. There was then a period of freedom for about a year, followed by two periods of recurrence which we were able to observe. The first of these latter periods lasted three months, and the gonococcus was isolated from the knee joints on two occasions.

TABLE 2

COMPLEMENT FIXATION TESTS MADE IN SECOND PERIOD OF RECURRENCE OF ARTHRITIS IN CASE JUST DESCRIBED

Date	Complement Fixation Gono- coccus	Wasser- mann	Date	Complement Fixation Gono- coccus	Wasser- mann
Jan. 18...	+++	—	April 29...	—	—
Jan. 25...	+++	—	April 30...	—	—
Jan. 31...	++	—	May 1...	—	—
Feb. 1...	++	—	May 2...	—	—
Feb. 7...	++	—	May 3...	—	—
Feb. 8...	++	—	May 4...	—	—
Feb. 15...	++	—	May 5...	+ weak	—
Feb. 20...	++	—	May 6...	—	—
Feb. 24...	+	—	May 7...	—	—
March 1...	+ weak	—	May 8...	+ weak	—
March 5...	+ weak	—	May 9...	+ weak	—
March 21...	—	—	May 10...	+	—
March 26...	—	—	May 11...	+ weak	—
April 1...	++	—	May 12...	—	—
April 5...	++	—	May 13...	++	—
April 15...	+ weak	—	May 14...	++	—
April 27...	—	—	May 15...	++	—
April 28...	—	—			

From January 18 to April 18 the patient was at home and reported at intervals for observation. The arthritis recurred at intervals of ten days and did not seem to be modified by inoculations of killed gonococci, with the possible exception that one interval of recurrence was fourteen, instead of the usual ten, days. On March 17 an unusually severe attack occurred in which there was also arthritis of sternoclavicular and shoulder joints. Following this, negative complement fixations were obtained. From April 27 to May 15 the patient was in the hospital and received no inoculations. Two attacks of arthritis during the patient's stay in the hospital (beginning April 27 and May 6, respectively) were characterized by the same sequence of events, i. e., on the first day pain in the knee, followed on the second day by effusion which increased for two or three days until the capsule of the joint was distended and contained 75-100 c.c. of fluid. If not aspirated, the fluid began to decrease on the fifth or sixth day and by the eighth day fluid was no longer demonstrable and the patient could walk without much discomfort.

Blood for tests was taken daily from the median vein. The complement fixation tests were made at the same time as those of blood from other patients whose records are included in this paper, and we are unable to account for the fluctuations observed in the reactions on any other ground than that the immune substances giving rise to the reaction varied in amount from day to day.

SUMMARY

A strong or moderate positive reaction obtained with adequate technical controls indicates the presence or recent existence of gonococcal infection.

A weak positive reaction has only a limited confirmatory value, and if the test is being made for diagnosis it should be repeated.

The reaction may change from positive to negative and from negative to positive during the course of an infection. This possibility should be taken into consideration in the interpretation of a negative result in a suspected case.

The reaction may become negative while gonococci are still present in the body and so a single negative reaction must be interpreted with great caution either in the diagnosis of infection or the determination of cure.

A METHOD OF TRANSMITTING BLOOD PARASITES *

JOHN A. KOLMER

(From the Department of Dermatological Research, Philadelphia Polyclinic and College for Graduates in Medicine, Philadelphia, Pennsylvania)

In chemotherapeutic researches involving the experimental infection of large numbers of white rats with *T. equiperdum*, *T. brucei*, *T. lewisi*, and *Sp. recurrentis*, it was found that the usual methods of obtaining a small amount of blood from an infected or seed rat by snipping the tail and bleeding into sodium citrate solution or defibrinating the blood with glass beads in a test tube were unsatisfactory because of the danger of contaminating the blood with various micro-organisms during the operation.

I have found the following method very serviceable. By this technic sufficient blood is obtained from the heart in an aseptic manner to infect a large series of rats without killing or seriously injuring the stock or seed animal. For the purpose of propagating a strain of trypanosomes by infecting one or two rats at regular intervals, the method has been found likewise serviceable, as a small amount of blood may be obtained aseptically and at frequent intervals without injury to the stock animal.

The seed rat is fastened to an operating board or held by a gloved assistant and the cardiac area determined by palpation, as when bleeding a guinea-pig from the heart. Owing to the rapid beating of the heart and thinness of the thoracic wall, this is easily and quickly determined.

One or two applications of tincture of iodine (10 percent) are made over the cardiac area to sterilize the hair and skin.

A test tube of sterile 1 percent sodium citrate in normal salt solution is warmed by heating in a Bunsen flame and a sufficient amount drawn carefully into a sterile 1 or 2 c.c. Record syringe, fitted with a medium-sized needle (No. 22), in such a manner as to avoid contamination. The 1 c.c. syringe is employed when few injections are to be made; for the purpose of infecting a larger series of rats, as thirty or more, a 2 c.c. syringe is used. The syringe is not entirely filled with the citrate-salt solution, but sufficient space is permitted

* Received for publication January 22, 1915.

for suction by withdrawal of the piston when the needle has been passed into the heart.

The skin over the cardiac area is drawn taut by the forefinger and thumb of the left hand without touching the site of puncture and the needle quickly entered into the heart, directed slightly upwards and toward the spine. No attempt is made to enter any particular chamber of the heart.

At once, blood flows into the syringe. The action may be facilitated by gentle suction, altho usually this is not necessary. When sufficient blood is obtained the needle is quickly withdrawn, the contents of the syringe are mixed by gentle agitation, and the animal returned to the cage. With heavily infected rats showing large numbers of trypanosomes or spirilli in every microscopic field, I secure a dilution of blood equivalent in density to a 1 percent suspension of erythrocytes. If the number of parasites in the blood of the seed rat is fewer in number, a correspondingly heavier emulsion is prepared. A heavy emulsion may be diluted by drawing into the syringe a sufficient amount of sterile normal salt solution (warmed) or by expelling the contents of the syringe into a sterile test tube and diluting as desired. Fresh rats are then infected by the intraperitoneal injection of 0.1-0.2 c.c. of the emulsion.

By this method the whole procedure is quickly conducted in a sterile manner and only in exceptional instances is the animal seriously injured or killed by the operation. It is important to use a sharp-pointed needle, and one not so large as to unnecessarily injure the heart and not so small as to hinder the flow of blood.

THE PRODUCTION, THROUGH IMMUNIZATION, OF SPECIFIC FERMENTS AGAINST BACTERIA AS DETECTED BY THE ABDERHALDEN TEST *

GEORGE H. SMITH

(From the Mulford Biological Laboratories, Glenolden, Pennsylvania.)

Since the appearance of Abderhalden's first papers concerning the principles and procedure involved in the serodiagnosis of pregnancy a voluminous literature has appeared. A great variety of physiological and pathological conditions have been investigated with discordant results in many cases. This fact is of itself significant in that it serves to emphasize the delicacy of the reaction, the many sources of error to which the worker is liable, and the importance of a uniform and highly perfected technic. In this connection it is noteworthy that many investigators have absolutely reversed their first opinions, as their skill in performing the test has increased.

The principle, as enunciated by Abderhalden, presupposes the production of a specific proteolytic ferment, elaborated in response to the parenteral introduction of a foreign protein.

That such a specificity of ferment production obtains in the case of tissue protein has been demonstrated conclusively in the opinion of many observers.¹ It must be admitted, however, that this opinion is not universal, in fact, there are those² who assert that no specificity exists. Others³ admit specificity in the case of most organ preparations but affirm non-specificity in the case of

* Received for publication January 25, 1915.

1. Abderhalden: München. med. Wehnschr., 1914, 61, p. 401.
Bruck: Ibid., 1913, 60, p. 1775.
Fischer: Deutsch. med. Wehnschr., 1913, 39, p. 2138.
Fuchs: München. med. Wehnschr., 1913, 60, p. 2230.
v. Gambaroff: Ibid., p. 1644.
Grey: Bull. Johns Hopkins Hosp., 1914, 25, p. 117.
Hirsch: Deutsch. med. Wehnschr., 1914, 40, p. 270.
Lampé: München. med. Wehnschr., 1914, 61, p. 463.
Lampé and Fuchs: Deutsch. med. Wehnschr., 1914, 40, p. 747.
Lampé and Papazolu: München. med. Wehnschr., 1913, 60, p. 1533.
Lowy: Jour. Am. Med. Assn., 1914, 62, p. 437.
Mayer: München. med. Wehnschr., 1913, 60, p. 2906.
Schiff: Ibid., 1914, 61, p. 768.
Veit: Berl. klin. Wehnschr., 1913, 50, p. 1241.
Wegener: München. med. Wehnschr., 1914, 61, p. 15.
Williamson: Jour. Obst. and Gynec. Brit. Emp., 1913, 24, p. 211.
2. Heilner and Petri: München. med. Wehnschr., 1913, 60, p. 1530.
Lange: Berl. klin. Wehnschr., 1914, 51, p. 785; Biochem. Ztschr., 1914, 61, p. 193.
Michaelis and v. Lagermarck: Deutsch. med. Wehnschr., 1914, 40, p. 316.
Mosbacher and Port: Ibid., p. 1410.
Oeller and Stephan: München. med. Wehnschr., 1914, 61, pp. 75, 579; Deutsch. med. Wehnschr., 1914, 40, p. 1557.
3. Bauer: Med. Klin., 1913, 9, p. 1797.
Frank, Rosenthal, and Biberstein: München. med. Wehnschr., 1913, 60, p. 1594.

some tissues, such as kidney tissue. Finally, there are those⁴ who maintain vigorously their belief in group reactions.

With respect to the application of the test, by far the greatest amount of work has been done on pregnancy, cancer, and mental disorders. From a review of the literature it would appear that its value as a diagnostic procedure in such cases is generally accepted.

With respect to the application of the reaction as a diagnostic method in infectious diseases, tuberculosis⁵ has received the greatest amount of attention. In this connection it must be admitted that the majority of workers report results far from satisfactory. In a few cases, however, the reports⁶ have been most encouraging, and in the opinion of some investigators tuberculosis in its various forms, degrees of severity, and duration can be differentiated by means of the nature of the substrate degraded. The sera from typhoid patients have been shown to contain a ferment capable of digesting typhoid bacilli.⁷ Reactions in the case of syphilitic sera⁸ are apparently specific.

Thus far, but one paper⁹ has appeared in which the limits of specificity of bacterial ferments have been considered. According to this report, the authors have demonstrated ferments which to a certain extent were specific, in that group reactions were obtained. The ferment produced by the inoculation of typhoid organisms was capable of degrading not only typhoid but also paratyphoid and the colon bacilli. Usually, such distantly related organisms as the typhoid bacillus and the staphylococcus could be differentiated, but even here these authors did not obtain uniformly specific results.

The experiment to be reported in this paper was designed to test the following hypothesis: The protein portions of bacteria which are capable of producing immunity, that is, the haptophorous groups of Vaughan, are essentially different in molecular constitution or configuration and when introduced parenterally, as in immunization, call forth ferments which are specific for the particular haptophore introduced.

In carrying out the work, the organisms employed were *Sta. aureus*, *Sta. albus*, streptococcus, pneumococcus, *B. influenzae*, and *M. catarrhalis*. These particular organisms were selected because of their application to another problem which was under consideration, yet

4. Kitchneff and Chingarewa: *Compt. rend. Soc. de biol.*, 1914, 76, p. 354.

Chingarewa and Kotschnéwa: *Russk. Vrach.*, 1914, 13, p. 101.

Singer: *München. med. Wchnschr.*, 1914, 61, p. 350.

5. Fränkel and Gumpertz: *Deutsch. med. Wchnschr.*, 1913, 39, p. 1585.

Gumpertz: *Beitr. z. Klin. d. Tuberk.*, 1914, 30, p. 201.

Jessen: *Ibid.*, 1913, 28, p. 489.

Krim: *Russk. Vrach.*, 1913, 12, p. 1502.

Lampé: *Deutsch. med. Wchnschr.*, 1913, 39, p. 1774.

Melikjanz: *Deutsch. med. Wchnschr.*, 1914, 40, p. 1369.

6. Fränkel: *Deutsch. med. Wchnschr.*, 1914, 40, p. 589.

Gwerder and Melikjanz: *München. med. Wchnschr.*, 1914, 61, p. 980.

Jessen: *Beitr. z. Klin. d. Tuberk.*, 1913, 28, p. 489.

Lampé: *Deutsch. med. Wchnschr.*, 1913, 39, p. 1774.

Wolff and Frank: *Berl. klin. Wchnschr.*, 1914, 51, p. 875.

7. Voelkel: *München. med. Wchnschr.*, 1911, 61, p. 349.

8. Reines: *Wien. med. Wchnschr.*, 1914, 64, p. 368.

Varney and Morse: *Jour. Michigan Med. Soc.*, 1914, 13, p. 515.

Voelkel: *München. med. Wchnschr.*, 1914, 61, p. 349.

9. Fekete and Gál: *Monatschr. f. Geburtsh. u. Gynäk.*, 1914, 39, p. 21.

it was felt that they would adapt themselves to this work since some of them are closely enough related to test effectively a comparatively high degree of specificity, while others are so distantly related that should only group reactions obtain, these also could be detected.

Suspensions of the organisms were prepared for use as vaccines in the production of immune sera in rabbits. The organisms were cultivated on either plain or blood agar. The growth was removed and was washed three times in physiological saline solution by means of the centrifuge. Bacterial counts of the resultant emulsions were made and the desired dilutions prepared. These were then held for one hour at temperatures ranging from 56 to 60 C.

The dilutions were as follows: *Pneumococcus*, *streptococcus*, *B. influenzae*, and *M. catarrhalis*, 12.5, 25, 50, 100, and 200 millions; of *Sta. aureus* and *Sta. albus*, 50, 100, 200, 400, and 800 millions. A series of mixtures containing each of the organisms in the same number as used for the individual dilutions was also prepared.

These dilutions were injected intravenously at intervals of four days. The animals used were apparently normal rabbits of both sexes and of approximately the same weight. Two rabbits received the immunizing course with each individual organism, and two with the mixture.

During the administration of the immunizing course, the bacteria, which were to serve as substrates, were grown. Agar bottles were inoculated with each of the organisms and, after a suitable incubation, the growth was removed by washing, was filtered through fine silk, and collected in centrifuge tubes. The process of washing by centrifugalization was repeated from three to seven times; those organisms which could be cultivated on plain agar received only three washings in physiological saline solution, while those which had to be grown on blood agar received a greater number. In the case of this last type, alternate washings in saline and water were given and, in this way, apparently all of the hemoglobin contained in the blood corpuscles, which were washed off with the bacterial growth, was removed. After the last washing a thick white emulsion was obtained, which was killed by heat as in the case of the vaccine preparations. The emulsions were then centrifugalized again, the supernatant fluid drawn off, and the bacterial residue placed in vacuum jars over sulphuric acid and dried. After thorough drying, the bacteria were pulverized as finely as possible and kept in the ice-box.

The dialyzing thimbles (Schleicher and Schüll No. 579A) were tested for impermeability with normal horse or rabbit serum. Those giving any trace of blue color when the dialysate was tested were discarded. The thimbles were also tested for impermeability against the *staphylococcus aureus*, but in no case was a positive reaction obtained where serum failed to give a positive result. Silk-peptone was used in testing for permeability. After the dialyzing thimbles had been tested they were kept in chloroform water under a layer of toluene and immediately before use they were boiled in several changes of distilled water. All of the glassware, corks, etc., were sterilized before use.

For purposes of testing, the animals were divided into two groups; one of the two rabbits receiving each kind of organism and one of those receiving the mixture being placed in each group. The rabbits of the first group were tested for ferment production about one week after the completion of the immunizing course. Those of the second group were tested about two weeks after the last immunizing injection. In obtaining the serum the animals were anesthetized,

the carotid artery exposed, and the blood collected in large tubes. Sterile precautions were observed throughout. As has been advocated by many observers,¹⁰ all food was denied the animals for at least eighteen hours before the withdrawal of blood.

When the serum had separated from the clot, it was drawn off and centrifugalized to free it from all cellular elements and then held in the ice-box until used. In no case did an interval greater than five hours elapse between the bleeding and the use of the serum. The control sera were obtained in a similar manner as the test sera, and fresh serum was used for control purposes with each day's tests.

In performing the test, 1.5 c.c. of serum and 10 mg. of the dried bacterial substrate were always used. The quantity of substrate was chosen arbitrarily but, since it was found that 10 mg. gave a very satisfactory result with positive sera, this amount was always used, altho probably a smaller quantity would have sufficed. Each serum under test was combined with the homologous substrate and also with the substrates prepared from each of the other organisms used in the experiment. The serum and substrate were now dialyzed against 20 c.c. of sterile, distilled water. Toluene was added both to the contents of the thimble and to the distilled water in the outside container.

Digestion and dialysis were allowed to proceed for sixteen hours at 37 C.

For control purposes the serum was dialyzed alone; the specific substrate alone, suspended in physiological salt solution; the substrate combined with normal serum; and normal serum alone.

At the completion of the period of dialysis 10 c.c. of the dialysate were removed, 0.2 c.c. of a 1 percent solution of ninhydrin was added, and the whole boiled for exactly one minute. Readings were taken at the end of one-half hour.

The results are summarized in the accompanying table.

DISCUSSION OF RESULTS

From the table it is evident that each of the sera, when combined with its homologous substrate, gave a very definite positive reaction. Catarrhalis sera reacted positively when combined with catarrhalis, and also in one serum slight reactions were obtained when streptococcus and the staphylococcus albus were used as substrates. Identical results were obtained with influenza serum. Serum 818 degraded influenza alone. Pneumococcus and streptococcus sera were specific, degrading none but the homologous substrates. One of the staphylococcus aureus sera gave a weakly positive reaction with streptococcus substrate. Staphylococcus albus degraded albus only.

10. Abderhalden and Lampé: *Ztschr. f. physiol. Chem.*, 1913, 85, p. 136.

Ball: *Jour. Am. Med. Assn.*, 1914, 62, p. 599; *Ibid.*, 63, p. 1169.

Grey: *Bull. Johns Hopkins Hosp.*, 1914, 25, p. 117.

Lowy: *Jour. Am. Med. Assn.*, 1914, 62, p. 437.

Scherer: *Berl. klin. Wchnschr.*, 1913, 50 p. 2183.

Schulz: *München. med. Wchnschr.*, 1913, 60, p. 2512.

TABLE 1

Serum	Date of Last Injection	Date Serum Was Tested	Substrates					Sta. Albus	None
			Catarrhalis	Influenza	Pneumococcus	Streptococcus	Sta. Aureus		
Catarrhalis.....{ 815 ♂ 816	April 22 April 22	April 29 May 6	++++ ++++	0 0	0 0	+	0 0	+	0 0
Influenza.....{ 817 ♂ 818	April 22 April 22	May 1 May 6	0 0	++++ ++++	0 0	+	0 0	+	0 0
Pneumococcus....{ 819 ♂ 826	April 24 April 24	May 2 May 7	0 0	0 0	++++ ++++	0 0	0 0	0 0	0 0
Streptococcus....{ 821 ♂ 822	April 24 April 24	May 2 May 7	0 0	0 0	0 0	++++ ++++	0 0	0 0	0 0
Sta. aureus.....{ 823 ♂ 824	April 26 April 26	May 3 May 12	0 0	0 0	0 0	+	++++ ++++	0 0	0 0
Sta. albus.....{ 825 ♀ 826	April 26 April 26	May 3 May 11	0 0	0 0	0 0	0 0	0 0	++++ ++++	0 0
Mixture.....{ 827 ♀ 828	April 26 April 26	May 4 May 11	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++	++++ ++++	0 0
Normal.....{	0 0	0 0	0 0	0 0	0 0	0 0	0 0
None.....{	0	0	0	0	0	0	

In the case of the rabbits, which received the immunizing course with the mixture, positive reactions were obtained with each of the different organisms. The controls in every case were perfect.

To explain the apparently non-specific results obtained in the combinations, catarrhalis-streptococcus and catarrhalis-staphylococcus albus, influenza-streptococcus and influenza-staphylococcus albus, and staphylococcus aureus-streptococcus, it may be said that in a subsequent experiment thirteen apparently normal rabbits have been tested for natural ferments against various strains of streptococci. In five of these rabbits positive reactions were obtained. This indicates that ferments which are capable of splitting streptococci, are present, perhaps as the result of accidental infection.

CONCLUSION

The only conclusion to be made at this time, disregarding entirely the theoretical aspects of the work, is that as the result of immunization ferments are produced which are specific for the organisms employed in the immunizing treatment.

THE PRODUCTION AND DETECTION OF SPECIFIC FERMENTS FOR THE TYPHOID-COLI GROUP*

GEORGE H. SMITH

(From the Mulford Biological Laboratories, Glenolden, Pennsylvania.)

The theoretical foundation for the specific ferment reaction of Abderhalden, as applied to physiological and pathological organic disturbances, and the methods of testing for the presence of these ferments have been so widely discussed in our medical literature that any consideration of them at this time is unwarranted. Further, any attempt to state the value of the reaction as a diagnostic aid or to solve the mechanism of the reaction, whether it be a true ferment action or otherwise, does not come within the scope of this paper. Without committing myself to any theory regarding the exact kind of bodies involved, I shall retain the nomenclature of Abderhalden and refer to the process as a specific or a non-specific ferment action, as the case may be. I wish to consider only the formation and manifestation of a specifically reacting substance elaborated by the body in response to the parenteral introduction of foreign protein, as in experimentally induced bacterial infection, or in immunization against bacteria.

In all the work previously reported, but few papers¹ have dealt with the application of the Abderhalden technic to bacterial infection where the specific invading organism itself served as fundament in the dialysis procedure. Generally, the subject has been approached from the angle of its significance as an aid to diagnosis, and in such work tissues which contain the causative organisms or have been altered by the infective processes have offered suitable and accessible materials as

* Received for publication January 25, 1915.

1. Abderhalden and Andryewsky: München. med. Wchnschr., 1913, 60, p. 1641.
Fekete and Gál: Monatschr. f. Geburtsh. u. Gynäk., 1914, 39, p. 21.
Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
Jessen: Ibid., 1913, 28, p. 489.
Kirschbaum and Köhler: Wien. klin. Wchnschr., 1914, 27, p. 837.
Massi: Riv. d'ig. e san. pubb., 1914, 25, p. 295.
Miessner: Deutsch. tierärztl. Wchnschr., 1913, 21, p. 417.
Voelkel: München. med. Wchnschr., 1914, 61, p. 349.

substrates. Particularly in tuberculosis and syphilis has this been true.² In the few cases where the organisms, or preparations of the organisms, such as the tuberculins and luetin, have been employed generally good results³ have been obtained, and the differential reactions obtained between tuberculous tissues and the tuberculins are considered of clinical importance.⁴

With respect to the degree of absolute specificity which exists in ferment production against bacterial fundamentals and which may be detected by means of the reaction, but little has been done, and the results are somewhat contradictory. Fekete and Gál⁵ reported that, in immunized animals, specificity of ferment production could be detected to the extent that staphylococcus could be differentiated from typhoid and colon bacilli. They were unable, however, to distinguish between typhoid and colon bacilli by means of the reaction. Complete details of their method are lacking in their report.

Kirschbaum and Köhler,⁶ using the sera of highly immunized horses, attempted to determine specifically of ferment action for the cholera, typhoid, paratyphoid, and dysentery bacilli. The results were very discordant, a condition which may be explained by the fact that the sera employed were from ten days to two months old.

In the preceding article, in my work with staphylococcus, streptococcus, pneumococcus, *M. catarrhalis* and *B. influenzae*, a complete specificity of ferment action was demonstrated. The present paper deals with a natural continuation of that work.

2. Baeslack: Jour. Am. Med. Assn., 1914, 62, p. 1002.
 Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
 Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
 Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
 Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
 Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
 Melikjaaz: Ibid., 1914, 40, p. 1369.
 Meyer-Betz: Ibid., p. 826.
 Reines: Wein. med. Wchnschr., 1914, 64, p. 368.
 Varney and Morse: Jour. Michigan Med. Soc., 1914, 13, p. 515.
 Voelkel: Ibid., p. 349.
 Wegener: Ibid., p. 15.
3. Abderhalden and Andryewsky: München. med. Wchnschr., 1913, 60, p. 1641.
 Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
 Gumpertz: Beitr. z. Klin. d. Tuberk., 1914, 30, p. 201.
 Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
 Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
 Krim: Russk. Vrach., 1913, 12, p. 1502.
 Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
 Melikjanz: Ibid., 1914, 40, p. 1369.
 Meyer-Betz: p. 826.
 Reines: Wein. med. Wchnschr., 1914, 64, p. 368.
4. Fränkel: Deutsch. med. Wchnschr., 1914, 40, p. 589.
 Gwerder and Melikjanz: München. med. Wchnschr., 1914, 61, p. 980.
 Jessen: Beitr. z. Klin. d. Tuberk., 1913, 28, p. 489.
 Lampé: Deutsch. med. Wchnschr., 1913, 39, p. 1774.
 Wolff and Frank: Berl. klin. Wchnschr., 1914, 51, p. 875.
5. Fekete and Gál: Monatschr. f. Geburtsh. u. Gynäk., 1914, 39, p. 21.
6. Kirschbaum and Köhler: Wien klin. Wchnschr., 1914, 27, p. 837.

To obtain the ferment-containing sera, rabbits were subjected to an immunizing treatment of five injections of *B. coli communis*, *B. coli communior*, paratyphoid (A), paratyphoid (B), and typhoids "Hopkins" and "Rawlings." The experiment has been conducted twice, and in both cases duplicate animals were immunized against each organism. Absolute uniformity in results was obtained. It may be said that not all supposedly normal rabbits are suitable for the work, since occasionally the serum of an animal will give positive reactions when combined with any substrate. This source of error was carefully controlled.

In the dialysis procedure only those thimbles which had proven suitable were used. The substrates employed were heavy emulsions of the bacteria. These were rendered free from ninhydrin-reacting substances by repeated boiling and washing. One cubic centimeter of the serum was combined with one cubic centimeter of the various substrates and allowed to dialyze for sixteen hours at 37 C., as in the Abderhalden technic. Sterility was considered essential. Serum and substrate controls were also necessary. At the end of the period of incubation, the dialysates were tested as usual.

The results speak for a high degree of specificity. The sera of rabbits immunized against the bacillus coli communis gave a positive reaction when dialyzed with communis as a substrate and a negative reaction when combined with the other organisms, even with so closely related an organism as the bacillus coli communior. Those sera derived from immunization with the bacillus coli communior degraded communior only. The reactions with the paratyphoids (A) and (B) were also specific, as no group reaction was manifested, nor any sign of interreaction between these sera and the individuals of the coli or typhoid groups. The rabbits which had been immunized with typhoid "Hopkins" furnished sera which did not react with either of the coli strains or with the paratyphoids, but which digested the substrates prepared from typhoids "Hopkins" and "Rawlings." Conversely, the sera of the animals immunized with typhoid "Rawlings" degraded both typhoids "Rawlings" and "Hopkins" but did not attack the others.

In addition, rabbits were subjected to immunizing treatment with a mixture of all of the strains. The results were as expected. Sera from these rabbits were able to effect a decomposition of all of the bacterial substrates. It is thus apparent that, under some conditions at least and within certain limits, a specificity of reaction occurs. Also, it is evident that the limit of specificity was reached with the typhoid sera. This indicates that the chemical composition or molecular configuration of the protein molecules in the two typhoid strains are suffi-

ciently similar to be degraded by one reacting body, and that there exists between the natures of the other bacterial proteins a dissimilarity so great that these proteins are incapable of digestion by one ferment (Table 1).

After determining that a true specificity of ferment production and action exists, experiments were conducted to determine the time relationships involved in the appearance of these ferments when the introduced bacterial protein existed in different physical states and the method of introduction was varied. Accordingly, living, killed, and killed sensitized preparations of typhoid "Rawlings" were administered in single injections of 50,000 millions. The methods of introduction were intravenous, intraperitoneal, and subcutaneous. Previous to the injection, trial bleedings were taken and tested to ensure the suitability of the animal for the experiment. During the period of experimentation all food was denied the animals, as enteral digestion has been cited as a cause for aberrant positive reactions.⁷

Following the introduction of the bacteria, bleedings were taken at intervals and tested according to the previously described method. The sera were always used within twelve hours of withdrawal from the animal as it has been repeatedly reported that a serum more than eighteen hours old is unsuited to the dialysis procedure.⁸

The results here reported are based on several determinations of each method employed, and it may be said that with a given method the time of appearance of the active principle never varied by more than three hours, and this only in the case of the animals injected subcutaneously. In general, the variation was less. In reading the results, all questionable reactions were considered negative and only the first well-defined, blue coloration of the dialysate was regarded as the time of appearance. This may not be the period of greatest intensity, for usually the reactions obtained with successive bleedings became increasingly deep in color up to a maximum. This is illustrated by the accompanying typical protocols:

7. Abderhalden and Lampé: *Ztschr. f. physiol. Chem.*, 1913, 85, p. 136.
Ball: *Jour. Am. Med. Assn.*, 1914, 62, p. 599; *Ibid.*, 63, p. 1169.
Grey: *Bull. Johns Hopkins Hosp.*, 1914, 25, p. 117.
Lowy: *Jour. Am. Med. Assn.*, 1914, 62, p. 437.
Scherer: *Berl. klin. Wehnschr.*, 1913, 1, p. 2183.
Schulz: *München. med. Wehnschr.*, 1913, 60, p. 2512.
8. Grey: *Bull. Johns Hopkins Hosp.*, 1914, 25, p. 117.
Lowy: *Jour. Am. Med. Assn.*, 1914, 62, p. 437.
Paine: *Boston Med. and Surg. Jour.*, 1914, 170, p. 303.
Scherer: *Berl. klin. Wehnschr.*, 1913, 1, p. 2183.

RABBIT 9, ♀

Bleeding A (normal).....	Serum control, 0
	Typhoid substrate, 0
	Serum + typhoid, 0
Injection of 50 thousand million killed, sensitized typhoid "Rawlings" intraperitoneally.	
Bleeding B (2 hours after injection).....	Serum control, 0
	Serum + typhoid, 0
Bleeding C (3 hours after injection).....	Serum control, 0
	Serum + typhoid, +
Bleeding D (4 hours after injection).....	Serum control, 0
	Serum + typhoid + + +
Bleeding E (5 hours after injection).....	Serum control, 0
	Serum + typhoid, + + + +
Bleeding F (6 hours after injection).....	Serum control, 0
	Serum + typhoid, + + + +
Bleeding G (30 hours after injection).....	Serum control, 0
	Serum + typhoid + + + +

RABBIT 10, ♀

Bleeding A (normal).....	Serum control, 0
	Typhoid substrate, 0
	Serum + typhoid, 0
Injection of 50 thousand million killed, sensitized typhoid "Rawlings" subcutaneously.	
Bleeding B (17 hours after injection).....	Serum control, 0
	Serum + typhoid, 0
Bleeding C (18 hours after injection).....	Serum control, 0
	Serum + typhoid, + +
Bleeding D (19 hours after injection).....	Serum control, 0
	Serum + typhoid + + + +
Bleeding E (20 hours after injection).....	Serum control, 0
	Serum + typhoid, + + + +
Bleeding F (22 hours after injection).....	Serum control, 0
	Serum + typhoid, + + + +
Bleeding G (24 hours after injection).....	Serum control, 0
	Serum + typhoid + + + +

DISCUSSION OF RESULTS

When the animals were treated intravenously, ferments appeared following an injection of live organisms in two hours, following an injection of killed, sensitized bacteria in one and one-half hours, and following an injection of killed organisms in three hours.

When treated intraperitoneally, the first appearance of ferments occurred after an injection of live typhoid in six hours, after an injection of killed, sensitized in three hours, and after an injection of killed typhoid in five hours.

When injected subcutaneously, a positive serum was first obtained with live typhoid after twenty-four hours, with killed, sensitized after eighteen hours, and with killed typhoid after thirty-six hours.

From this work it appears that the intravenous method of administration is most rapid in its results, and the subcutaneous gives the slowest response (Chart 1).

TABLE 1
SHOWING RESULTS OF EXPERIMENTS

Serum		Date of Last Injection	Date Serum Was Tested	B. coli communis	B. coli communior	Paraty- phoid (A)	Paraty- phoid (B)	Typhoid "Hopkins"	Typhoid "Rawlings"	None
B. coli communis.....	876	♀	Dec. 14	+++++	0	0	0	0	0	0
	877	♂	Dec. 14	+++++	0	0	0	0	0	0
B. coli communior....	880	♂	Dec. 14	0	+++++	0	0	0	0	0
	881	♀	Dec. 14	0	+++++	0	0	0	0	0
Paratyphoid (A).....	882	♀	Dec. 14	0	0	+++++	0	0	0	0
	883	♀	Dec. 14	0	0	+++++	0	0	0	0
Paratyphoid (B).....	884	♂	Dec. 14	0	0	0	+++++	0	0	0
	885	♂	Dec. 15	0	0	0	+++++	0	0	0
Typhoid "Hopkins"...	886	♀	Dec. 15	0	0	0	0	+++++	+++++	0
	887	♂	Dec. 15	0	0	0	0	+++++	+++++	0
Typhoid "Rawlings"...	888	♀	Dec. 15	0	0	0	0	+++++	+++++	0
	889	♂	Dec. 15	0	0	0	0	+++++	+++++	0
Mixture	892	♂	Dec. 15	+++++	+++++	+++++	+++++	+++++	+++++	0
	893	♀	Dec. 15	+++++	+++++	+++++	+++++	+++++	+++++	0
Normal	0	0	0	0	0	0	0
None	0	0	0	0	0	0	0

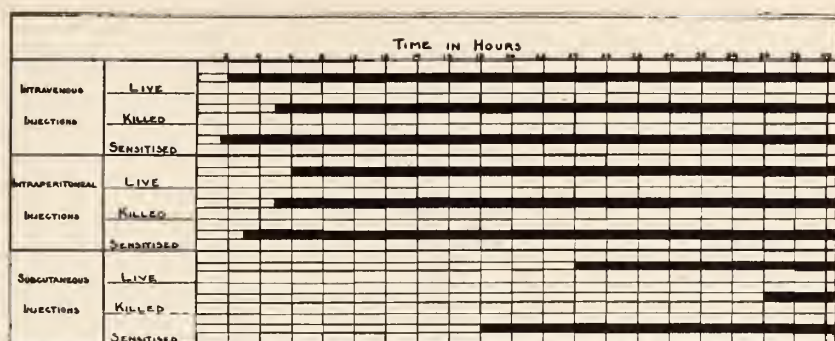


Chart 1.—Showing time relations between intravenous, intraperitoneal and subcutaneous injections.

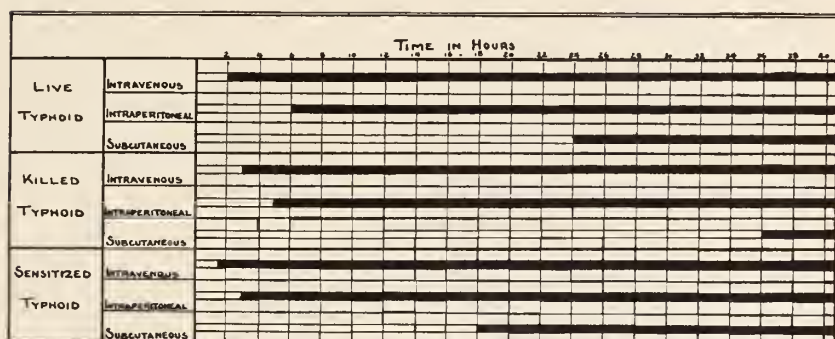


Chart 2.—Showing the relation of kind of material employed to rate of formation of ferments.

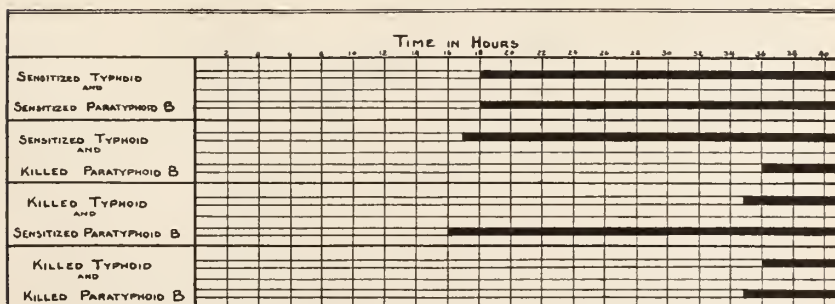


Chart 3.—Showing time relations between simultaneous injections of two kinds of bacilli.

With regard to the type of material employed, the killed, sensitized was most potent in inducing a rapid formation of ferment (Chart 2).

In continuation and substantiation of this conclusion, subcutaneous injections were given simultaneously of killed typhoid and killed paratyphoid (B), of killed typhoid and sensitized paratyphoid (B), of sensitized typhoid and killed paratyphoid (B), and of sensitized typhoid and sensitized paratyphoid (B). When killed organisms of both types were injected, ferments for paratyphoid (B) appeared in thirty-three hours and for typhoid in thirty-six hours. When killed typhoid and sensitized paratyphoid (B) were employed, the serum degraded paratyphoid (B) after sixteen hours and typhoid after thirty-five hours. Reversing the natures of the two types injected, ferments for typhoid were demonstrable after seventeen hours, and for paratyphoid (B) after thirty-six hours. With the final combination in which both types were sensitized, the sera gave positive results with both substrates after eighteen hours (Chart 3).

But one inference can be drawn from this, namely, that the previous treatment of the bacteria with immune serum renders them more susceptible to assimilation by the body, and thus enables them to bring about a more rapid formation of the active principle of parenteral digestion.

CONCLUSIONS

As a result of the parenteral introduction of bacterial protein, some change occurs in the serum of the treated animal, specific ferment formation or otherwise, which causes it to give a positive reaction when combined with its homologous substrate and negative reactions when dialyzed with others.

The method of administration, in a measure, controls the time of appearance of this property; intravenous injections cause the most rapid formation of ferments.

The physical, or chemical state of the material introduced, controls to a degree the time of appearance of this property; the killed, sensitized preparation is most efficient.

The value of the reaction as a means of bacterial differentiation is suggested.

Under proper conditions, the reaction may prove to be an aid to the diagnosis of infectious diseases and may prove of value in indicating the most efficient means to employ in the vaccine therapy of such conditions.

A STUDY OF THE CORRELATION OF THE AGGLUTINATION AND THE FERMENTATION REACTIONS AMONG THE STREPTOCOCCI *

I. J. KLIGLER

(From the Department of Bacteriology, Columbia University, and Department of Public Health, American Museum of Natural History, New York City)

Bacterial classification has passed through three distinct phases. First, we had the purely morphological classification based on size, shape, and form of the organisms and the colonies they produce. Later, when the inadequacy of a division founded on these characters alone was realized, various physiological reactions were added with the hope of obtaining a better grouping. Finally, pathogenic and immunity reactions were introduced in the differentiation of some groups. The later workers, however, did not always attempt to base their classifications on the correlation of these groups of characters, but recommended instead different systems depending on the types of reactions they happened to have observed. This created a considerable amount of confusion.

This state of confusion is especially notable among the streptococci. This group has from the very beginning been the subject of a great deal of controversy.

REVIEW OF THE LITERATURE

The discussion of the unity and multiplicity of the streptococci is too well known to bear repetition. Nor is it necessary to go further into the detailed history of the study of this group. Splendid critical summaries of the literature are given by Winslow,¹ and more recently by Hopkins and Lang.² In general, it will be noted that the first classification was a morphological one—*longus*, *brevis*, and *media*. It was soon found, however, that this separation was artificial and unreliable. Consequently Schottmüller³ proposed a classification based on the action of these organisms on blood. Subsequently Gordon⁴ and Andrewes and Horder⁵ suggested a grouping based on fermentation reactions which promised to be scientifically tenable as well as practically valuable. This work was followed in this country by that of Winslow

* Received for publication January 29, 1915.

1. Jour. Infect. Dis., 1912, 10, p. 285.

2. Ibid., 1914, 15, p. 63.

3. München. med. Wchnschr., 1903, 20, p. 849.

4. Rept. Med. Off. to Local Gov. Bd., Great Britain, 1902, 32, p. 421.

5. Lancet, 1906, 171, p. 708.

and his co-workers¹ and Rogers,⁶ who succeeded in bringing to light some valuable information on the streptococci of milk, cow and horse, and normal man. The pathogenic cocci, however, were not studied as carefully until recently when Hopkins and Lang² and, about the same time, Lyall⁷ published thorough studies of this group. Hopkins and Lang used the fermentation tests only, while Lyall also utilized the hemolysin test, determined by a new volumetric method.

With the increasing volume of research a great body of apparently contradictory results has been published. However, a careful analysis of these data reveals the fact that the various workers, tho employing somewhat different technics, arrive at similar conclusions. Most authors agree that the streptococci generally ferment the sugars in a definite gradation, the simpler sugars being attacked first, then the complex, then the glucosid (salicin), and finally the alcohols. Organisms attacking a disaccharid generally ferment also the monosaccharids, and those fermenting a glucosid will also ferment the disaccharids. This has been aptly termed by Winslow the "metabolic gradient." There is also a general agreement that morphology, or length of chain, does not correlate with any other character. The value of the hemolysin test, on the other hand, is still undecided, tho it appears to be significant among the pathogenic streptococci. For the last mentioned group, Andrewes and Horder, Hopkins and Lang, and Lyall agree that salicin, raffinose, mannite, and inulin are the significant sugars, and that the group which generally ferments salicin (consequently also dextrose, lactose, and saccharose) but fails to ferment mannite, raffinose and inulin, and usually produces hemolysis is a definite species corresponding to the streptococcus pyogenes. Lyall uses the hemolysin test for the primary division, while the others use the sugars as the basis. A comparison of their tables reveals however a substantial agreement. Less certainty exists regarding the mildly pathogenic raffinose and mannite fermenters. These types have been vaguely classed as either viridans, salivarius, or fecalis, but as yet no satisfactory systematic division has been made.

For the non-pathogenic forms from human and animal origins, certain facts also have been definitely established. Winslow and Palmer,⁸ confirmed by Fuller and Armstrong,⁹ showed that lactose was significant in distinguishing the horse forms from those of human and bovine origins, as the former generally failed to ferment that sugar. The bovine forms are generally found to ferment raffinose, while the human ferment mannite.

None of the workers mentioned attempted to utilize the immunity reactions for the classification of this group. Aronson¹⁰ and Marmorek¹¹ showed that the serum of one strain of pyogenic streptococcus may give a considerable amount of protection against other strains. They also showed that agglutinins produced by one strain will agglutinate other strains, usually in higher concentrations. These authors seem to think that while the agglutination reaction may be relied on to separate streptococci from pneumococci, it is of little value for differentiation of types within the group.

6. Jour. Ag. Research, 1914, I, p. 491.

7. Jour. Med. Research, 1914, 30, p. 487.

8. Jour. Infect. Dis., 1910, 7, p. 1.

9. Ibid., 1913, 13, p. 1.

10. Berlin klin. Wehnschr., 1902, 39, p. 1006.

11. Ibid., 39, p. 299.

Besredka and Dopter¹² used the complement fixation test on a series of scarlet fever streptococci without positive results. Faix and Mallein,¹³ on the other hand, claim to have obtained cross fixation with nine strains of throat streptococci from cases of scarlet fever. Swift and Thro¹⁴ studied the complement fixation and conglutination tests and found fixation specific for each of the five strains tested, while the latter test tended to bring out the group relationship.

Obviously, there must be some explanation for these conflicting results. It seemed likely that, by comparing the immunity reactions of the streptococci with their other properties, some light might be shed on this perplexing subject. In this paper, the following two points were taken up: Do the immunity reactions separate the pathogenic streptococci into distinct groups? Do the groups so formed correspond with those obtained by the use of the fermentative and other characters, i. e., is there a definite correlation between the immunity reactions and the other properties of these organisms?

Only cultures isolated from some pathogenic source were studied. In all, sixty strains were used. The tests employed were morphology; hemolysis; fermentation of lactose, saccharose, salicin, raffinose, mannite and inulin, and agglutination. Later, absorption tests were carried on parallel with the agglutinations, and in a few instances, complement fixation tests were made. A survey of the literature indicated however that the agglutination reaction was the one most likely to yield satisfactory results, and it was therefore used throughout the investigation.

METHODS

Morphology.—Smears were made from twenty-four-hour glucose broth cultures, and the average length of the chain was observed. My results are in accord with those of the other authors. In a general way, the salicin fermenters gave long chains, but frequently the chains of mannite fermenters were equally long. It was also noticed that the raffinose and mannite fermenters generally gave more abundant growths in glucose broth. The actual observations were not sufficiently uniform to warrant repetition here.

Hemolysis.—Tests for hemolysin production were made by streaking a loop-full of a twenty-four-hour culture of the organism grown on North's medium on the surface of a blood agar plate, containing 1 c.c. of defibrinated rabbit's blood to about 10 c.c. of agar. The

12. Ann. de l'Inst. Pasteur, 1904, 18, p. 373.

13. Presse méd., 1907, 15, p. 777.

14. Arch. Int. Med., 1911, 7, p. 24.

results were recorded under three heads: H, hemolysis; G, green colonies with or without slight hemolysis; and N, gray or brown colonies showing no action on hemoglobin. The results were not always constant, and in two or three cases it was uncertain in which group the organism belonged. On the whole, the reaction was uniform. An interesting observation, the constancy of which was not determined, is the fact that many of the raffinose fermenters gave green colonies accompanied by a slight zone of hemolysis, while the mannite fermenters usually gave a green to a greenish brown colony without any hemolysis. This reaction was merely noted and no significance can be claimed for it.

Fermentation.—The fermentation tests were made on two different occasions after an interval of about two months. The first test was made in meat infusion broth containing 1 percent peptone. It was found in agreement with Hopkins that a broth containing 2 percent peptone gave more uniform results, and so this medium was used for the second series of titrations. In both instances, the sugar broths were inoculated with a loop-full of a twenty-four-hour culture on North's medium. This was found more desirable than inoculations from a broth culture because no foreign, split protein substances of an unknown nature were introduced into the sugar broths, and also for the reason that the organisms have a greater viability and produce more abundant growth on the semi-solid than they do in the liquid medium. The sugar broths were thus seeded with a large number of vigorous cells, and consequently more constant results were obtained.

The non-uniformity of fermentative reactions attributed to the streptococci are due to the vigor of the inoculum (the culture) and the character of the new environment (the medium). It is well known that a vigorous culture inoculated into an unfavorable medium will give but sparse growth and that a weak culture even if inoculated into a favorable medium will often give no growth at all. It naturally follows that attenuated organisms, placed in an unsuitable environment, will generally fail to develop. A number of authors have demonstrated the fact that a culture, even in a fairly vigorous condition, when transferred to a fresh medium will pass through a definite period of retarded development before vigorous growth at the maximum rate begins. In the case of the streptococci, this is probably what happens. The organism, when placed in a medium

containing a glucosid or a higher sugar finds itself in an unnatural environment because the broth itself does not contain the substances favorable to active growth. Consequently, a period of "lag," as Penfold¹⁵ names it, sets in during which the organism divides very slowly, while at the same time disintegration processes must be taking place. When weak cells or small numbers of vigorous cells are introduced into the medium containing the higher sugars, the cells die before they are able to exercise their latent power to utilize the sugar or alcohol for energy purposes.

This idea was confirmed in a number of ways. Inoculations were made into sugar broths from a broth culture, shown to contain living cells, by streaking on North's medium, and the former, when tested after twenty-four hours, were found to be absolutely sterile. Approximately the same amount of inoculum placed into salicin broth made up with 1 and 2 percent peptone, respectively, gave negative results in the former and active fermentation in the latter. On the other hand, two strains tested at thirty-day intervals for four months on the same batch of medium (kept tubed and capped on ice to prevent evaporation) gave uniform results. The tests were qualitative, but they all tend to show that the variable fermentations are frequently to be attributed as much to faulty methods as to the variability of the organisms. That the different cells of the same strain may vary somewhat in their power to ferment a certain sugar is an established fact.

The cultures were incubated for three days at 37 C. and the acidity titrated with $n/20$ NaOH, with phenolphthalein as an indicator. The results of the second series of titrations were similar to those of the first, except that in a number of instances salicin was fermented in the second series only. This is in all probability due, as stated above, to the more favorable character of the former medium.

Agglutination.—In all cases, the blood of the rabbits to be immunized was tested against the culture used in the immunization to ascertain the absence of agglutinins.

The first and second inoculations were made with killed twenty-four-hour glucose broth cultures. Subsequent inoculations were made at five-day intervals with increasing doses of living cultures. The size of the dose depended on the condition of the animal. Five to six

15. Jour. Hyg., Cambridge, 1914, 14, p. 215.

inoculations were generally sufficient to give a fairly high titer. In a number of cases, notably with cultures of the mannite fermenting groups, no immune substances could be detected in a dilution higher than 1:20, even after eight inoculations.

The method suggested by Hiss¹⁶ of growing the culture in carbonate broth was found to be very satisfactory. The cultures can be stored on ice and used repeatedly. With this method, spontaneous clumping is reduced to a minimum.

The agglutinations were conducted in standard tubes using dilutions of the serum of 1:20, 1:50, 1:100, and 1:200. Controls of the homologous culture of salt water were always made. The tubes were incubated at 37 C. for two hours and then put in the ice chest for eighteen to twenty-four hours. The results were recorded as +, ++, +++, and ++++ according as agglutination occurred in one, two, three, or four dilutions. Cultures that repeatedly clumped spontaneously in the salt water control were considered negative.

Parallel absorption tests were made with the last three of the four sera used. The results did not differ materially from those obtained with the agglutination test. A great deal of difficulty was experienced in absorbing the agglutinins, even with the homologous culture.

Complement Fixation.—In two cases, where the agglutinins in the serum were rather low, complement fixation tests were made with an emulsion of the organism and antiformin extract, respectively, as antigens. The results showed that the complement fixing substances were not more active than the agglutinins. The result is indicated by the following protocol:

Culture	Amount of Serum in c.c. (1-10)	Amount of Antigen in c.c.	Complement in c.c.	Inhibition	Agglutination	
					1:20	1:50
20	0.5	0.1	0.5	++	+	—
	0.2	0.1	0.5	++		
	0.1	0.1	0.5	+		

Control tests were made with antigen and serum alone, respectively, with negative results.

RESULTS

The results obtained with the various tests are shown in Table 1. With the exception of three or four cultures, the break between acid and non-acid producers is very sharp. All cultures producing 1 percent acid or over were considered positive; those below, negative.

TABLE 1
GIVING IN DETAIL THE REACTIONS OF THE DIFFERENT STRAINS

No.	Source	Lac- tose*	Saccha- rose*	Sali- cin*	Raffi- nose*	Man- nite*	Inu- lin*	Hemolysis			Agglutination with Serum			
								H	G	N	9	38	43	54
1	Blood, sepsis.....	1.5 +	2.5 +	3.0 +	0.0 —	3.0 +	0.0 —	+	—	—	—	—
2	Blood, septis.....	2.5 +	2.6 +	3.0 +	0.7 —	0.5 —	0.0 —	+	++++	—	—	—
3	Pus, abscess.....	2.8 +	2.7 +	0.8 —	3.0 +	0.4 —	0.0 —	..	+	..	—	—	—	—
4	Pus, mastoid.....	2.9 +	2.8 +	2.8 +	0.4 —	0.6 —	0.0 —	+	+	—	—	—
5	Blood, sepsis.....	1.5 +	4.0 +	3.6 +	1.0 +	0.4 —	0.0 —	..	+	..	—	—	—	—
6	Pericarditis	3.5 +	3.8 +	4.0 +	0.8 —	3.8 +	0.0 —	..	.	+	—	—	—	—
7	Blood (same pa- tient as No. 3)	2.4 +	2.6 +	died	—	—	—	—
8		2.5 +	2.8 +	2.8 +	0.9 —	0.6 —	0.0 —	+	—	+	—	—
9	Blood, sepsis.....	2.5 +	2.9 +	4.0 +	0.6 —	0.2 —	0.0 —	+	++++	—	—	—
10	Peritonitis	+	3.2 +	3.2 +	0.7 —	3.6 +	0.0 —	+	—	—	—	—
11	Pus, abscess.....	+	3.0 +	3.0 +	0.3 —	0.4 —	0.0 —	+	—	—	—	—
12	Pus, knee abscess.	2.4 +	2.6 +	3.0 +	0.4 —	0.4 —	0.0 —	+	++++	—	—	—
13	Erysipelas	2.5 +	2.7 +	3.0 +	0.3 —	0.4 —	0.0 —	+	..	.	—	—	—	—
14	Blood, sepsis.....	2.9 +	2.6 +	4.0 +	0.6 —	0.4 —	0.2 —	+	++	—	—	—
15	Septis, puerperal..	2.5 +	3.0 +	3.0 +	0.1 —	0.2 —	0.2 —	+	++++	—	—	—
16	Sepsis, puerperal..	3.0 +	3.1 +	2.8 +	0.0 —	0.6 —	0.0 —	+	—	—	—	—
17	Blood, cellulitis...	2.5 +	3.2 +	2.8 +	0.1 —	0.6 —	0.1 —	+	—	—	—	—
18	Pus, abscess.....	2.8 +	3.8 +	2.6 +	0.1 —	0.2 —	0.0 —	..	+	..	—	—	—	—
19	Blood, sepsis.....	—	+	+	—	—	—	+	—	—	—	—
20	Blood, sepsis.....	2.6 +	2.6 +	3.2 +	0.4 —	3.4 +	0.0 —	+	—	—	—	—
21	Blood, sepsis.....	2.4 +	2.9 +	3.5 +	0.5 —	0.2 —	0.0 —	+	+	—	—	—
22	Blood, sepsis.....	1.5 +	2.5 +	3.0 +	0.2 —	0.0 —	0.0 —	+	—	—	—	—
23	Pleuritis	2.7 +	2.3 +	2.3 +	2.4 +	0.4 —	0.0 —	..	+	..	—	+	—	—
24	Pleuritis	2.4 +	2.5 +	3.8 +	2.5 +	0.0 —	0.2 —	..	+	..	—	+	—	—

* The numbers indicate the percent of normal acid.

TABLE 1.—(Continued)

No.	Source	Lac- tose*	Saccha- rose*	Sali- cin*	Raffi- nose*	Man- nite*	Inu- lin*	Hemolysis			Agglutination with Serum			
								H	G	N	9	38	43	54
25	Peritonitis	2.9 +	3.2 +	3.6 +	0.0 —	0.0 —	0.1 —	+	—	—	—	—
26	Appendicitis	2.5 +	2.5 +	2.5 +	0.2 —	2.5 +	0.0 —	+	—	—	—	—
27	Pus, abscess.....	2.9 +	2.8 +	3.0 +	0.6 —	0.5 —	0.0 —	+	—	—	—	—
28	Pus, empyema.....	2.6 +	2.7 +	2.8 +	0.8 —	0.8 —	0.0 —	+	—	—	—	—
29	Acute tonsillitis (tonsil)	2.5 +	2.7 +	2.7 +	0.4 —	0.5 —	0.0 —	+	++++	—	—	—
30	Sputum, bron- chitis	2.0 +	2.5 +	3.5 +	4.1 +	0.4 —	0.0 —	..	+	..	—	—	—	—
31	Pus, otitis media..	3.8 +	3.0 +	0.6 —	4.8 +	0.7 —	0.0 —	..	+	..	—	—	—	—
32	Acute tonsillitis (tonsil)	3.2 +	3.6 +	3.8 +	3.2 +	0.3 —	1.3 +	..	+	..	—	++++	—	—
33	Pus, abscess.....	3.0 +	2.5 +	2.4 +	0.6 —	0.4 —	0.0 —	+	++++	—	—	—
34	Liver abscess.....	2.8 +	2.7 +	3.0 +	0.4 —	0.2 —	0.6 —	+	—	—	—	—
35	Pus, abscess pros- tate	3.5 +	4.0 +	3.8 +	0.6 —	3.5 +	0.0 —	..	+	..	—	—	—	—
36	Pus, abscess.....	2.5 +	3.2 +	3.0 +	0.4 —	0.5 —	0.0 —	+	++++	—	—	—
37	Stool	3.2 +	3.5 +	5.0 +	5.3 —	4.0 +	0.0 —	..	+	..	—	—	—	—
38	Tonsil, normal....	3.3 +	4.4 +	5.0 +	3.2 +	0.2 —	0.0 —	..	+	..	—	++++	—	—
39	Gum, pyorrhea....	4.0 +	2.8 +	1.2 +	1.0 +	0.3 —	0.0 —	..	+	..	—	—	—	—
40	Stool	3.2 +	2.9 +	5.0 +	0.3 —	3.9 +	0.0 —	..	+	..	—	—	..	—
41	3.3 +	3.6 +	4.2 +	2.8 +	3.1 +	0.0 —	+	—	—	—	—
42	Gland, scarlet fever	2.3 +	2.7 +	2.7 +	0.3 —	0.6 —	0.0 —	+	+++	—	—	—
43	Throat, scarlet fever	2.6 +	2.8 +	2.6 +	0.1 —	3.3 +	0.0 —	+	—	—	++++	—
44	Throat, scarlet fever	2.2 +	3.0 +	2.0 +	1.5 +	2.7 +	0.0 —	+	—	—	++	—
45	Liver, abscess.....	3.7 +	3.8 +	5.0 +	0.3 —	0.2 —	0.0 —	+	—	—	—	—
46	Cow, rapplides.....	1.2 +	3.1 +	0.6 —	0.4 —	0.2 —	0.0 —	+	—	—	—	—
47	Cow, mastitis.....	2.9 +	3.0 +	0.4 —	0.2 —	0.2 —	0.0 —	+	—	—	—	—
48	Nose, scarlet fever	2.0 +	2.6 +	2.9 +	0.7 —	0.7 —	0.0 —	+	+	—	—	—

* The numbers indicate the percent of normal acid.

TABLE 1.—(Continued)

No.	Source		Lactose*	Saccharose*	Salicin*	Raffinose*	Mannite*	Inulin*	Hemolysis			Agglutination with Serum			
									H	G	N	9	38	43	54
49	Throat,	scarlet	1.3 +	2.4 +	0.7 —	2.4 +	0.0 —	0.0 —	..	+	..	—	—	—	—
50	Gland,	scarlet	2.6 +	2.3 +	3.1 +	2.8 +	0.5 —	0.0 —	+	+	+	—	—
51	Septic sore throat,		2.6 +	2.9 +	2.8 +	0.7 —	0.0 —	0.0 —	+	—	—	—	—
52	Septic sore throat,		2.9 +	3.2 +	2.9 +	0.6 —	0.0 —	0.0 —	+	++++	—	—	—
53	Erysipelas	2.8 +	2.5 +	3.8 +	0.7 —	0.0 —	0.0 —	+	+++	—	—	—
54	Blood,	endocarditis	3.0 +	3.1 +	3.5 +	3.0 +	3.0 +	0.2 —	..	+	..	—	—	—	+++
55	Blood,	endocarditis	1.4 +	3.1 +	3.0 +	3.5 +	0.6 —	0.0 —	..	+	..	—	++	—	—
56	Blood,	endocarditis	3.4 +	4.0 +	3.5 +	4.2 +	0.3 —	0.0 —	..	+	..	—	++	—	—
57	Blood,	endocarditis	3.2 +	3.0 +	3.9 +	3.2 +	3.6 +	0.0 —	..	+	..	—	—	—	—
59	Pus, abscess.....		2.3 +	2.5 +	2.8 +	0.0 —	0.1 —	0.0 —	+	—	—	—	—
	Pus, abscess.....		2.2 +	2.6 +	3.0 +	0.3 —	0.1 —	0.0 —	+	+++	—	..	—

The interesting point in this study is the correlation of the agglutination reactions with other characters. Four agglutinating sera having titers between 800 and 1,000 were selected. These sera represented, respectively: A salicin fermenting, hemolytic strain (Strain 9); a raffinose fermenting, green colony producing strain (Strain 38); a strain fermenting salicin and mannite but not raffinose and producing no change on the blood (Strain 43); a strain fermenting salicin, mannite, and raffinose and producing green colonies on blood agar (Strain 54).

Tables 2-5 present a summary of the agglutination tests with these sera. The salicin serum agglutinated fifteen out of thirty-one of the salicin fermenting strains, twelve of them in dilutions of 1:50 or over, while it agglutinated only one raffinose fermenting strain in a dilution of 1:20, and failed to agglutinate any of the other fermentative types. Similar results were obtained with the raffinose serum, seven out of thirteen raffinose fermenters being agglutinated, four of them in dilutions of 1:50 or over. Of the others, only one salicin fer-

TABLE 2

AGGLUTINATION OF VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY A STRAIN FERMENTING SALICIN ONLY (STRAIN 9)*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	15	12	11	8
Raffinose fermenters	13	1	0	0	0
Mannite fermenters	9	0	0	0	0
Mannite and raffinose fermenters	4	0	0	0	0

* This table shows that dilutions greater than 1:20 made the serum specific for salicin fermenters—tho even some of these were eliminated by this dilution.

TABLE 3

AGGLUTINATION OF THE VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY A STRAIN FERMENTING SALICIN AND RAFFINOSE*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	1	0	0	0
Raffinose fermenters	13	7	4	2	2
Mannite fermenters	9	0	0	0	0
Mannite and raffinose fermenters	4	0	0	0	0

* This table shows that the serum was specific for raffinose fermenters in dilutions greater than 1:20, tho nearly half of the agglutinated strains were eliminated by the higher dilutions.

TABLE 4

AGGLUTINATION OF VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY STRAIN FERMENTING SALICIN AND MANNITE AND NOT RAFFINOSE*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	0	0	0	0
Raffinose fermenters	13	0	0	0	0
Mannite fermenters	9	1	1	1	1
Mannite and raffinose fermenters	4	1	1	0	0

* This table shows that the serum was specific for the homologous strain except in one instance where a strain, fermenting both raffinose and mannite, was agglutinated in a dilution of 1:50, indicating that the types fermenting mannite alone and those fermenting mannite and raffinose are closely related.

TABLE 5

 AGGLUTINATION OF VARIOUS FERMENTATIVE TYPES BY THE SERUM PRODUCED BY A STRAIN
 FERMENTING SALICIN, RAFFINOSE, AND MANNITE*

Group	Number of Strains Tested	Number of Strains Agglutinated by a Dilution of the Serum			
		1:20	1:50	1:100	1:200
Salicin fermenters	31	0	0	0	0
Raffinose fermenters	13	0	0	0	0
Mannite fermenters	9	0	0	0	0
Mannite and raffinose fermenters	4	1	1	1	1

* This table shows that the serum was specific for the homologous strain. Both this and the previous table tend to confirm the view that the mannite fermenters are less homogeneous than the other groups.

TABLE 6

CORRELATION OF FERMENTATION GROUPS WITH AGGLUTINATION*

Fermentation Group	Number of Cul- tures in Each	Cultures Agglutinated by Serum Produced by							
		Salicin Fermenter		Raffinose Fermenter		Mannite Fermenter		Mannite and Raffinose	
		Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent
Salicin	31	15	48.4	1	3.2	0	0	0	0
Raffinose	13	1	7.7	7	53.9	0	0	0	0
Mannite	13	0	0	0	0	1	15.4	1	7.7

* In this table, as well as in Table 7, all the cultures agglutinated by the different sera are included irrespective of the dilutions used, because this tends to bring out more strikingly the group relationships of the different strains.

TABLE 7

CORRELATION OF HEMOLYTIC GROUPS WITH AGGLUTINATION

Hemolytic Group	Number of Cul- tures	Cultures Agglutinated by Serum Produced by							
		Hemolytic Strain		Viridans Strain 1		Non-hemolytic Strain		Viridans Strain 2	
		Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent
Hemolysis	29	10	34.5	2	7.0	1	3.5	0	0.0
Green colony, slight or no hemolysis	17	0	0.0	6	35.3	0	0	1	5.8
No reaction....	12	5	41.7	0	0.0	1	8.3	0	0.0

menting strain was agglutinated and that in a dilution of 1:20. The mannite sera were specific for the respective homologous strains.

In no case was cross agglutination obtained in a dilution higher than 1:20. The two instances of such cross agglutination would indicate that this concentration does not bring out the specificity of the serum, tho a relatively greater number of homologous than heterologous strains were agglutinated in that dilution. Dilutions of

TABLE 8
CORRELATION OF FERMENTATIVE CHARACTERS WITH OTHER PROPERTIES

Group	Author	Number of Cultures Used	Sources												Size of		
			Suppuration		Septicemias		Acute Throat Infections		Endocarditis, etc.		Chronic Throat, etc.		Saprophytes		L.		M.
			Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number
Salicin fermenting strains	K.	31	14	47	12	40	3	10	0	0	0	0	1	3	25	48	6
	H.	52	22	42	10	19	5	10	4	8	8	15	3	6			
	L.	100	26	28	26	28	29	32	1	1	0	0	10	11			
Average	84 percent						16 percent								
Raffinose fermenting strains	K.	13	2	15	2	15	0	0	4	31	2	15	3	22	8	40	4
	H.	20	1	5	0	0	0	0	4	20	6	30	9	45			
	L.	55	1	3	1	3	13	39	1	3	4	12	14	52			
Average	30 percent						70 percent								
Mannite fermenting strains	K.	13	3	23	3	23	0	0	3	23	0	0	4	30	2
	H.	15	4	26	1	7	0	0	5	34	0	0	5	33			
	L.	37	0*	0	7	47	2	13	3	20.0	2	13	1	7			
Average	46 percent						54 percent								

* The grouping as to source is admittedly arbitrary and may not meet the approval of pathologists, but it is convenient for my purpose. Under acute throat infections are included septic sore throat and acute tonsillitis. Under endocarditis are classed also rheumaticus strains and those from pyorrhea. Under chronic throat are included chronic tonsillitis, bronchitis, and mild inflammations. In the saprophyte group are included all strains from normal throats, milk, etc. Under the heading, Size of Chain, L = longus; M = media; B = brevis. Under Hemolysis, H = hemolysis; G = green colony or

the sera of 1:50 or over are specific for the group; no cross agglutinations were obtained.

The correlation between agglutination and fermentative and hemolytic powers, respectively, is shown in Tables 6 and 7. At first sight the agreement is not very striking. Even if the non-specific agglutinations (agglutinations in a dilution of 1:20) are included, a correlation of only 50 percent is obtained with two of the fermentative groups and of only 35-40 percent with the hemolytic groups. A closer

analysis brings to light some interesting points. The serum produced by the salicin fermenter agglutinates only cultures of the same fermentative characters, except in one instance where slight agglutination was obtained with a raffinose fermenting strain. The same holds true of the serum produced by the raffinose fermenter, which agglutinates members of its own division. The sera produced by the mannite fermenting strains, on the other hand, remain practically specific for

TABLE 8
CORRELATION OF FERMENTATIVE CHARACTERS WITH OTHER PROPERTIES

Chain			Hemolysis								Agglutination								Fermentation	
			H.		G.		N.				S +.		R +.		S +. M +.		S +. M +. R +		Salicin	Raffin
M.	B.																		Percent. +	Perce
Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent		
11.6	18	34.6	23 43 74	74 82.5 74	1 — 16	3 — 16	6 7 20	19 13.4 20	15 — —	48 — —	1 — —	3 — —	0 — —	0 — —	0 — —	0 — —	100 100 100	0 0 0		
				77		9.5		17.4											100	0
20	8	40	1 — 1	7.7 — 2	11 38	84.7 68	1 16	7.7 29	1 — —	7.7 — —	7 — —	54 — —	0 — —	0 — —	0 — —	0 — —	77 60 73	0 0 0		Mann Perce
				3		72		25											70	0
14	12	86	4 3 9	31 20 24	5 .. 14	38 .. 38	4 11 14	31 73 38	0 — ..	0 — ..	0 — ..	0 — ..	2 — ..	15	1 — .	8 — ..	100 93 78	30 20 13		Raffin Perce
				26		38		36											86	18

methemoglobin reaction; N = no changes in the hemoglobin. The column showing size of chain is included to bring out the absence of any definite correlation between this character and the others.

In all cases, the percentages are determined on the basis of the number of cultures used in the particular test. This does not always bring out the true relationships, but this is a difficulty one generally has to contend with in comparing the results of different authors. Where comparable data exists, as in the case of Lyall's and my own hemolytic results, a close agreement is noted between the fermentation reactions and the source of the salicin fermenters.

their respective homologous strains. Therefore, while the agglutination reaction is apparently too specific for the differentiation of broad groups and not all the cultures of the same fermentative characters are agglutinated by their respective sera at the same time, the fact that a serum of one fermentative type was capable of agglutinating only strains of that particular group and not the others would indicate that the fermentation reactions tend to divide the streptococci into broad, distinct species. It is likely that by means of the agglu-

tion reaction these fermentative species may be further subdivided into varieties, similar to those obtained by Dochez and Gillespie among the pneumococci.

The correlation of agglutination with hemolysis is not so marked as that with fermentation. Thus 41 percent (five strains) of the indifferent strains were agglutinated by the serum of the hemolytic strain, and only 8 percent (one strain) by that of the indifferent strain. On the other hand, the agglutination correlation within the groups was about 35 percent for hemolytic and viridans strains, respectively. It seems that a primary division on the basis of hemolysis, as suggested by Lyall, is not warranted by the actual biologic relationships of the groups as indicated by the agglutination tests. Of course, it is possible that a better correlation might be obtained with his quantitative procedure.

On the whole, it is evident that the agglutination tests shed a considerable amount of light on the significance of the fermentative properties of the streptococci and on the importance of certain sugars for their differentiation into broad, specific types. The salicin group, as pointed out, has already been recognized by most workers as a well-defined, specific type. My observations are entirely in accord with these results. There is less agreement on the definiteness of the raffinose and mannite types. The agglutinations indicate that the organisms fermenting raffinose and not mannite constitute a fairly definite group. The mannite fermenters, on the other hand, form the center of a variable and ill-defined group, including mainly the saprophytic or mildly pathogenic forms.

The plausibility of this classification is strengthened when one recasts the data obtained by Hopkins, Lyall, and myself so as to bring out the correlation between the three fermentative types suggested and the various other characters. These results are shown in Table 8. There is a very substantial agreement between the results obtained by the different authors, despite the fact that non-uniform methods of recording them renders them at times incomparable. Hopkins, for instance, gives full data as to source and length of chain of his strains but fails to mention the methemoglobin, or green colony reaction. Lyall, on the other hand, gives full information as to the source of his salicin fermenting strains but furnishes meager data concerning the origin of the others. Nevertheless, certain facts stand out prominently. Thus, of the salicin fermenters 84 percent of which were

obtained from suppuration, septicemia, and acute throat infection, 77 percent hemolyzed blood, while only 9 percent produced green colonies. Of the raffinose fermenters, only 30 percent were found in acute infections and of these about two-thirds were from acute throat conditions. Seventy percent came from subacute and chronic infections, or saprophytic sources. Hemolysis was rare, while 72 percent produced green colonies. About 70 percent of these strains also fermented salicin. The mannite group again stands out as indefinite. The strains divide about equally between acutely and mildly pathogenic and saprophytic types. Tested on hemoglobin, they present almost equal numbers of hemolytic, viridans, and indifferent types. About 86 per cent of the strains also ferment salicin, while only about 20 percent attack raffinose. It seems to me that the evidence tends strongly to corroborate the conclusions arrived at from the study of the agglutination reactions.

SUMMARY AND CONCLUSIONS

Sixty strains of streptococci from various pathological conditions were studied with respect to their agglutinative and fermentative properties.

The agglutination reaction was not found to separate the streptococci into large groups. However, by its correlation with the fermentation reactions, the probable relationship of these types is indicated.

The agglutination tests tend to show that a division of the streptococci on the basis of hemolysis is not warranted, whereas a separation according to the fermentation reactions appears to coincide more closely with their natural relationship.

The groups suggested are:

Str. pyogenes.—Salicin fermenters, which do not ferment raffinose or mannite, are generally hemolytic, and strongly pathogenic.

Str. salivarius.—Raffinose fermenters, usually ferment salicin but do not ferment mannite, generally produce a green colony on blood agar, and usually cause subacute and chronic infections.

Str. fecalis.—Mannite fermenters, generally ferment salicin, rarely ferment raffinose, and are variable in their reaction to blood and in their pathogenicity.

THE DIAGNOSTIC VALUE OF INTRACUTANEOUS INJECTION OF DIPHTHERIA TOXIN (SCHICK REACTION)*

GEORGE H. WEAVER AND LORETTA K. MAHER

(From the Memorial Institute for Infectious Diseases, Chicago)

By means of the Schick intracutaneous reaction, individual susceptibility and immunity to diphtheria may be readily recognized with a fair degree of accuracy. This has been turned to account in the selection of persons requiring immunization in the presence of exposure to diphtheria and in the sparing of those, already protected, from further annoyance and discomfort and from needless sensitization to horse serum. This reaction has also facilitated the study of the immunizing effects of injections of diphtheria toxin-antitoxin mixtures, as advocated by Behring. Schick has also employed the reaction to determine the quantity of diphtheria antitoxin required to bring a person infected with diphtheria into the condition of an immune individual and so to determine the doses of antitoxin required in various sorts of cases. It has also been valuable in estimating the relative efficiency of single and multiple injections of antitoxin and of injections made in various ways.

The technic of the test as devised by Schick¹ and followed by others is simple and readily applied. First, the minimum fatal dose (M. L. D.) of the diphtheria toxin is determined for a guinea-pig of 250 gm. The toxin is then so diluted that each cubic centimeter contains 0.2 of the M. L. D. Of this dilution, 0.1 c.c. (0.02 M. L. D.) is injected into the skin. The injection is made with a very fine, sharp, short-pointed needle, and a successful injection results in the production of a white, blister-like, punctate-appearing elevation. The traumatic disturbance disappears in a few hours, and if there is antitoxin in the blood of the injected person no further changes occur. This is regarded as a negative result and indicates that the person is immune to infection by diphtheria bacilli. If the person has very little or no diphtheria antitoxin in the blood, the disappearance of the traumatic

* Received for publication January 30, 1915.

1. München. med. Wchnschr., 1913, 60, p. 2608.

disturbance is followed by a gradually increasing redness and infiltration at the site of injection. This reaches its height in twenty-four to forty-eight hours, persists several days, and gradually fades leaving a brownish pigmentation and slight desquamation. This is designated a positive reaction. Sometimes individuals who have antitoxin in the blood show some local redness and infiltration. This is not due to the toxin injected since it may follow the injection of the same amount of toxin combined with many times the amount of antitoxin necessary to neutralize it. These so-called false reactions usually come on more rapidly than the true ones, the redness is less sharply outlined, the local infiltration is more marked, and they disappear in two to four days with no subsequent desquamation and little or no pigmentation.

During the later part of the year 1914 in the Durand Hospital, we have given the intracutaneous toxin injection in all cases of diphtheria and suspected diphtheria at the time of admission, and we have employed it in all incoming interns and nurses. The technic has been that indicated. The injections have been made into the skin of the outer side of the middle third of the arm. Those reactions have been considered positive in which a redness and some induration appeared after twenty-four hours and persisted for six to ten days with succeeding pigmentation and scaling. Whenever slight redness appeared inside of twenty-four hours and rapidly subsided with no later pigmentation and scaling it was considered a false reaction. Some individuals, who were immune to diphtheria, exhibited marked redness in twenty-four hours which quickly subsided leaving no further signs. In cases giving a false reaction, we have found that a control injection in the opposite arm of the same amount of toxin combined with 1,000 times the amount of antitoxin required for neutralization, as advised by Groër and Kassowitz,² sometimes also calls forth a false reaction. Groër and Kassowitz conclude from a large experience that a negative result from the toxin injection in adults signifies that there is antitoxin in the blood, but that a positive result does not indicate an absence of antitoxin except when the reaction with the toxin-antitoxin mixture is negative. They found that a considerable number (47.5 per cent) of mothers of new-born children react positively from the intracutaneous injection of diphtheria toxin in spite of the presence of normal antitoxin in the blood. In these individuals, toxin neutralized by antitoxin in vitro also causes positive reactions. They designate

such reactions "paradoxical." Similar reactions with the toxin-antitoxin mixture occurred in mothers with no antitoxin in the blood, so that 56 percent of mothers give paradoxical reactions. They found that 11.2 percent of new-born infants are insusceptible to specific diphtheria toxin inflammation even in a poverty of antibodies, so that a negative result with the intracutaneous test in these cases must not be considered final. In a number of the women they obtained no reaction by injections of dilute solutions of broth and horse serum, or of pure antidiphtheritic serum. To explain these paradoxical reactions they have advanced certain theories. One group of theories rests upon the assumption that the individual for some reasons is unable to neutralize diphtheria toxin within the body; the other class of theories attempts to explain the reaction as one of allergy.

Many of our tests have been controlled by estimates of the antitoxin in the blood of the injected individuals, employing Römer's method. No estimates were made for less than 0.04 unit of antitoxin per cubic centimeter of blood. That this method measured the antitoxin accurately was shown by controlling the results by tests made according to the usual method for testing the potency of antidiphtheritic serum.

Of ten normal young adults, interns and nurses, six gave negative and four positive reactions. In each of these ten individuals the blood serum was tested for its antitoxin content. In each of the four who reacted positively the antitoxin was less than 0.04 unit per cubic centimeter of blood. Of the six cases giving negative reactions, one had 0.04 unit, two had 0.125 unit, and three had 2 units per cubic centimeter of blood. The proportion of those giving positive Schick reactions is rather high, but the number of observations is relatively small for comparison. Park, Zingher, and Serota³ in one hundred and twenty-four persons of 15 years and over obtained positive results in thirty-one, or 25 percent.

Schick¹ has collected two hundred and sixty-four observations in persons from 5 to 15 years of age, 50 percent of whom gave positive reactions. In adults, the proportion of positive reactions was still smaller. The proportions of positive reactions occurring at various ages, as determined by Schick and by Park, Zingher, and Serota, correspond very closely. From the second to the fifth year positive reactions occurred in about 65 percent of the cases observed. Groër

and Kassowitz² found that 84 percent of new-born children and their mothers have a considerable amount of normal diphtheria antitoxin in the blood serum. Two of the nurses in our series who gave negative results had received diphtheria antitoxin for diphtheria two and four years before, respectively. Nurses giving positive reactions are immunized with diphtheria antitoxin at intervals during their stay in the hospital.

The test was applied in fourteen cases of angina which proved not to be diphtheria. Twelve of these cases were tonsillitis, ten of which reacted negatively and two positively. In two of these cases giving negative reactions, diphtheria bacilli were present in throat cultures. In one of them the blood serum contained 0.04 unit and in the other 5 units of antitoxin per cubic centimeter. In four cases giving negative reactions, the simultaneous injection of antitoxin may have influenced the result. In a case of Vincent's angina, the reaction was negative, the blood serum containing 0.04 unit of antitoxin per cubic centimeter. In a case of secondary syphilis with a positive Wassermann, the Schick test resulted negatively and the blood serum contained fifteen units of antitoxin per cubic centimeter. The value of the test as a diagnostic aid in cases of angina of uncertain character is apparent. It often enables a differentiation to be made between diphtheria and tonsillitis and other infections in which the patient is a diphtheria bacillus carrier. The latter cases could hardly be benefited by diphtheria antitoxin. Park and Zingher⁴ have found the Schick test of value as a diagnostic measure in cases with a purulent or sanious nasal discharge showing the diphtheria bacillus, in which it is difficult to decide whether the case is a carrier or a beginning diphtheria. A negative result excludes diphtheria. Reiche⁵ has reported twenty-three cases which clinically and bacteriologically were Vincent's angina, but in which diphtheria bacilli were also found in the throat. Some of these he believed to have been diphtheria bacillus carriers. The diagnostic value of the Schick test in such cases is very evident.

Four cases admitted as diphtheria bacillus carriers were tested and all gave negative results. In three there was a history of slight sore throat some time before admission. In one case the antitoxic content of the blood was six units per cubic centimeter, in another it was fifteen units per cubic centimeter. In a third case the antitoxin in the blood

4. Proc. New York Path. Soc., 1914, 14, p. 151.

5. Med. Klin., 1914, 10, p. 1345.

increased from five to ten units per cubic centimeter during the first week in the hospital. The fourth case came to the hospital just about the time of the recovery of the sore throat. At this time there was less than 0.04 unit of antitoxin per cubic centimeter of blood. In the two following weeks it rose to thirty units per cubic centimeter. These observations seem to indicate a pronounced production of antitoxin in mild cases of diphtheria which recover spontaneously. Other observers have found that diphtheria bacillus carriers have relatively abundant antitoxin in the blood. These facts speak in favor of the theory advanced by German writers which ascribes the frequent presence of normal antitoxin in the blood to a former, slight infection by diphtheria bacilli, often so mild as to have failed to attract attention. Park and Zingher⁴ have pointed out that the normal antitoxin may be due to other factors, as they often found all the children of a family to react similarly, giving either positive or negative results. In these instances the influences of former mild family infections would be excluded with difficulty.

Groër and Kassowitz⁶ have thoroughly studied the normal diphtheria immunity in man and concluded that the antidiphtheritic substance found in normal serum is identical in physical and chemical properties with immune antitoxin.

In ten cases of acute diphtheria we tested the blood serum for antitoxin at the time of admission before any antitoxin had been administered. In every instance it was below 0.04 unit per cubic centimeter. This agrees with the conclusions of all observers that an absence of normal antitoxin is essential for infection by diphtheria bacilli. The amount of antitoxin required for protection is usually stated to be 0.03 + unit per cubic centimeter of blood, but Behring believes that 0.01 unit per cubic centimeter is sufficient. Park, Zingher, and Serota³ injected a child, weighing 35 pounds, with 10 units of antitoxin and twenty-four hours later the Schick reaction was prevented. The antitoxin in the blood could scarcely have been more than 0.01 unit per cubic centimeter. In twelve cases of diphtheria the toxin injection was given on admission and antitoxin was injected immediately afterward. Four cases received 5,000 units each, two reacting positively and two negatively; one received 6,000 units and reacted negatively; three received 10,000 units each, two reacted positively and one negatively; four received 20,000 each, two reacted

positively and two negatively. Thus, it appears that the reaction is prevented or modified by sufficient antitoxin given simultaneously. In some of the reactions recorded as negative there was a very slight, modified, or abortive reaction. In all cases of diphtheria tested after the disease had been controlled by antitoxin, the cutaneous reaction was negative.

CONCLUSIONS

In normal persons a negative result from the intracutaneous injection of diphtheria toxin constantly indicates the presence in the blood of diphtheria antitoxin with a consequent immunity to diphtheria, at least for the time being. A typical positive result points to an absence of antitoxin and a resulting susceptibility to infection by diphtheria.

In the presence of exposure to diphtheria, immunization by injections of antitoxin is not indicated in persons who give negative reactions, but only in those who give positive reactions.

Intracutaneous injections of diphtheria toxin are valuable in separating cases of infection by diphtheria bacilli from cases of angina and rhinitis due to other causes. They also serve to distinguish cases which are diphtheria from those which are only bacillus carriers.

Diphtheria bacillus carriers usually develop relatively large amounts of antitoxin in the blood.

In the acute stage of diphtheria, before any antitoxin has been injected, the patient's blood contains little or no antitoxin.

In cases of acute diphtheria, full doses of antitoxin given simultaneously with the toxin injection frequently modify or completely inhibit the cutaneous reaction.

The Journal of Infectious Diseases

PUBLISHED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. 16

May, 1915

No. 3

THE INFLUENCE OF AN OXIDIZING SUBSTANCE (SODIUM IODOXYBENZOATE) ON IMMUNE REACTIONS *

AARON ARKIN

(From the Otho S. A. Sprague Institute and the Departments of Pathology of the University of Chicago, Chicago, and West Virginia University, Morgantown, West Virginia.)

A review of the influence of chemical substances on immune reactions shows us that little has been done to determine the relation of oxidation to antibody formation, altho some of the results suggest a definite relationship between the two. Certain substances, which have the property of accelerating oxidation, have been found to have a stimulating effect on the production of antibodies, for example, the colloidal metals studied by Bossan and Marcelet¹ and Robin and Bordet,² which stimulate the production of opsonins. Strychnin has a stimulating effect on phagocytosis in vitro³ as has also sodium iodoxybenzoate, an active oxidizing agent,⁴ which stimulates the production of lysin in the dog, as shown by Hektoen.⁵ Arsenic compounds also have a stimulating effect, as demonstrated by Agazzi⁶ for agglutinin, Friedberger and Masuda⁷ for hemolysin, and Strubell⁸ for opsonin.

*Received for publication February 10, 1915.

1. Gaz. d. hôp., 1908, 81, p. 1227.
2. Comp. rend. Acad. d. sc., 1904, 138, p. 783.
3. Arkin: Jour. Infect. Dis., 1913, 13, p. 408.
4. Ibid., 1912, 11, p. 427.
5. Tr. Chicago Path. Soc., 1911, 8, p. 138.
6. Ztschr. f. Immunitätsf., 1909, 1, p. 736.
7. Therap. Monatsh., 1911, 25, p. 288.
8. Berl. klin. Wehnschr., 1912, 49, p. 1076.

We have evidence that substances which depress oxidations have an inhibitory effect on immune reactions. Numerous experiments have demonstrated that chloroform, ether, and alcohol have a detrimental effect on the natural defenses against infection (Rubin⁹) and interfere with the production of antibodies (Graham¹⁰). Morphine, potassium cyanid, and chloral have a depressing effect on phagocytosis (Arkin³), and chloral also prevents anaphylactic shock in the guinea-pig.¹¹ The fact that guinea-pigs protected by chloral do not become antianaphylactic indicates that this drug influences the mechanism of the allergic reaction. Some chemical substances which have no effect on the processes of oxidation in the body seem to have no marked effect on immune reactions. The antipyretics,¹² caffeine,³ and many other chemical substances have no influence on the production of antibodies.

In addition to this relationship between the effect of chemical substances on oxidation and their influence on the production of antibodies, we find a striking similarity between the activity of oxidizing ferments and the action of immune body and complement.¹³ In both, three things are essential: A substance of a ferment nature; a substance on which it can act; and a substance which enables the ferment to act on the substrate and so to cause hydrolysis, oxidation, or some other type of reaction. Also, the ferment and the substrate are usually more specific to each other than to the third body which is simpler in nature, such as an alkali, an acid, a peroxid, or complement in immune serum. Finally, there is a similarity between the oxidizing ferments and immune sera in regard to thermostability. When an immune serum is heated to 55 C. the complement is destroyed but the immune body is unaffected. Likewise, when a vegetable extract is heated to 55 C. the peroxid is destroyed, but the ferment or peroxidase is untouched, and the inactivated juice can be reactivated by adding peroxid, just as the serum is reactivated by adding unheated serum containing complement.

Substances which are active oxidizing agents may influence the oxidation in the tissues by acting as catalysers. This action would not be due to the small amount of oxygen which they contain, but more likely to their catalytic effect, just as certain metals hasten oxidative processes. Most oxidizing agents cannot be used intravenously because they cause methemoglobin formation, gas embolism, or are not reduced

9. Jour. Infect. Dis., 1904, 1, p. 425; Jour. Amer. Med. Assn., 1907, 48, p. 1432.

10. Jour. Infect. Dis., 1911, 8, p. 147.

11. Banzhaf and Famulener: *Ibid.*, 1910, 7, p. 577.

12. Kentzler and v. Benzur: *Ztschr. f. klin. Med.*, Berl., 1909, 67, p. 242.

13. Moore and Whitley: *Biochem. Jour.*, 1909, 4, p. 136.

in the body. For this reason, little is known regarding the reaction of cells to increased oxidation.

Sodium iodoxybenzoate, an organic peroxid which contains physiologically active oxygen and can be injected intravenously, offers an opportunity of studying the influence of oxidation on immune reactions. By increasing the intravital oxidations in the tissues it might have an effect on antibody production. The preparation of this compound has been discussed by me in a previous publication.⁴ It is made from iodbenzoic acid, which is first oxidized to iodosobenzoic acid then to iodoxybenzoic acid. It contains two atoms of oxygen attached to the iodine in the molecule, thus possessing 11.43 percent of available oxygen. The oxygen can be determined by its power of liberating iodine from potassium iodide in acid solution.

The action of these substances was first studied by Heinz¹⁴ in 1899. By the use of them, he attempted to study the effect of nascent iodine in the body. Our knowledge of the pharmacological action of sodium iodoxybenzoate we owe to Loevenhart¹⁵ and his pupils who demonstrated that this compound oxidizes hemoglobin to oxyhemoglobin and can furnish oxygen for the peroxidase reaction by oxidizing phenolphthalin to phenolphthalein in the presence of blood. It causes a fall of blood pressure on intravenous injection by action on the vasomotor center, and it also acts on the respiratory center, producing apnea. These effects can be attributed to the oxygen attached to the iodine atom in the molecule, for iodbenzoic acid has no such action. Furthermore, the compound antagonizes the effect of hydrocyanic acid on the respiratory center. F. Jahn¹⁶ has recently made a careful study of the effect of iodosobenzoic acid.

Sodium iodoxybenzoate has little effect on the blood. It causes a slight leukocytosis in about twenty-four hours after intravenous injection. This increase is in polymorphonuclears. The red blood cell count and hemoglobin are practically unchanged, as shown in the results, following the injection of 5 c.c. of a N/20 solution of sodium iodoxybenzoate intravenously into a rabbit.

Time	Hemoglobin Percent	Leukocytes	Erythrocytes
Before injection.....	90	15,000	6,100,000
6 hours after injection.....	85	18,000	5,900,000
24 hours after injection.....	85	20,000	5,700,000
90 hours after injection.....	90	16,000	6,000,000
120 hours after injection.....	90	16,000	6,000,000

14. Virchows Arch., 1899, 155, p. 44.

15. Loevenhart and Grove: Jour. Phar. and Exper. Therap., 1911, 3, pp. 101, 131.

16. Arch. f. exper. Path. u. Pharmacol., 1914, 76, p. 16.

These results agree with those of Loevenhart in his study of the pharmacologic action of sodium iodoxybenzoate.

That sodium iodoxybenzoate possesses oxygen in active form was demonstrated by me¹⁷ in a study of its germicidal action toward various bacteria. These experiments showed that sodium iodbenzoate has very little bactericidal power as compared with that of the oxygen-containing substances. When the colon bacillus is used, the substances stand in the following relation to one another:

Substance	Oxygen Percent	Concentration Required to Kill	Relative Bac- tericidal Action
Sodium iodobenzoate.....	0.0	N/10 (2.7%)	1
Sodium iodosobenzoate.....	6.06	N/1000 (0.028%)	100
Sodium iodoxybenzoate.....	11.43	N/2000 (0.015%)	200

We see that the germicidal action of the oxygen-containing substances is dependent upon the physiologically active oxygen attached to the iodine. I found differences in the relative bactericidal value of sodium iodosobenzoate and iodoxybenzoate toward different organisms. These differences do not argue against the proposition that the germicidal action is due to the oxidizing action. A great many instances of specificity in oxidations with the simplest chemicals may be pointed out. If A and B each destroy C and D by oxidative processes, the fact that C is more sensitive to A than B does not indicate that D will also be more sensitive to A than B; indeed, the reverse is just as likely to be true. This statement holds whether C and D are living microorganisms or relatively simple chemicals.

We have further evidence in the action of similar organic peroxides in which the location of the oxygen atom or atoms in the benzene ring determines the activity of the compound as an oxidizing agent. The symmetrical organic peroxides with substitutions on both sides are inactive, the asymmetrical substitution products are active. There is here a promising field for chemotherapy, the discovery of organic peroxides with specific oxidizing properties or with specific activity toward certain bacteria. In this connection, I have found that the germicidal action of sodium iodoxybenzoate toward the four organisms which I studied varies inversely with the catalase value of the bacteria, i. e., the organism richest in catalase is least susceptible to the oxidizing agent. These results, which will be published later, would suggest that the enzyme catalase of bacteria protects them against active oxidizing agents and that the specificity may be due in part to the catalase content of the various bacteria studied.

17. Jour. Phar. and Exper. Therap., 1911, 3, p. 145.

Not only does sodium iodoxybenzoate have a marked germicidal action dependent upon its oxygen content, but I have found that it also has a marked stimulating action on phagocytosis *in vitro*.⁴ This property is not possessed by sodium iodbenzoate which contains no oxygen. The opsonin of the serum is probably rendered more active by the oxygen-containing substance. Furthermore, the fact that potassium cyanid and many other substances, which exert an inhibitory effect on oxidations (alcohol, ether, chloroform, chloral, etc.), have a marked depressing effect on phagocytosis suggests a relationship between oxidation and the process of phagocytosis. If we agree with Verworn that ameboid movement is produced by decreased surface tension resulting from the introduction of oxygen into the living substance, we can readily see how the stimulating effect of sodium iodoxybenzoate on phagocytosis is produced.

That sodium iodoxybenzoate stimulates the production of antibody in immunized animals was first demonstrated by Hektoen.⁵ He observed that dogs receiving the drug intravenously produced a greater amount of hemolysin than control animals or animals receiving sodium iodbenzoate. This demonstration led me to study the effect of sodium iodoxybenzoate on the production of various antibodies (hemolysin, agglutinin, and opsonin) in order to demonstrate, if possible, a relationship between the process of intravital oxidation and the mechanism of antibody formation.

ACTION OF SODIUM IODOXYBENZOATE ON THE PRODUCTION OF HEMOLYSIN

Rabbits immunized with ox corpuscles have been used in this work. Each rabbit was injected intravenously with 2.5 c.c. of washed ox corpuscles diluted with sufficient salt solution per kilogram of body weight. Fresh animals of about similar weights were selected (variation was not more than 75 gm.). Some of the rabbits then received an intravenous injection of 5 c.c. of neutral 1 percent sodium iodoxybenzoate one hour after the injection of the washed ox corpuscles. The sodium iodoxybenzoate used throughout this work was prepared by me and contained 11.2 percent of active oxygen when titrated with sodium thiosulphate. The control animals and those injected with the drug were then bled and the hemolytic value of the serum determined; 5 percent suspension of fresh ox corpuscles was used. The blood was obtained from the ear and the serum inactivated by heating to 56 C. for thirty minutes in a water bath. The dilutions were then made in sterile test tubes to which sufficient salt solution was added to make 1 c.c. in each. To each tube was added 0.05 c.c. guinea-pig complement, obtained by bleeding two guinea-pigs just before the experiment, and also 1 c.c. of a 5 percent suspension of fresh ox corpuscles. Controls were made with complement and corpuscles, amboceptor and corpuscles, and salt solution and corpuscles. The tubes were incubated at 37 C. for two hours and then placed in the ice-chest for twenty-four hours.

Chart 1 shows that sodium iodoxybenzoate has a stimulating effect on the production of specific hemolysin in rabbits injected with ox corpuscles, just as Hektoen found for dogs injected with goat corpuscles. The results indicate that the production of antibody is in some way connected with the process of oxidation, for a substance which is a physiologically active oxidizing agent stimulates antibody formation. Furthermore, sodium iodbenzoate, which contains no active oxygen, does not possess this property. This action of sodium iodoxy-

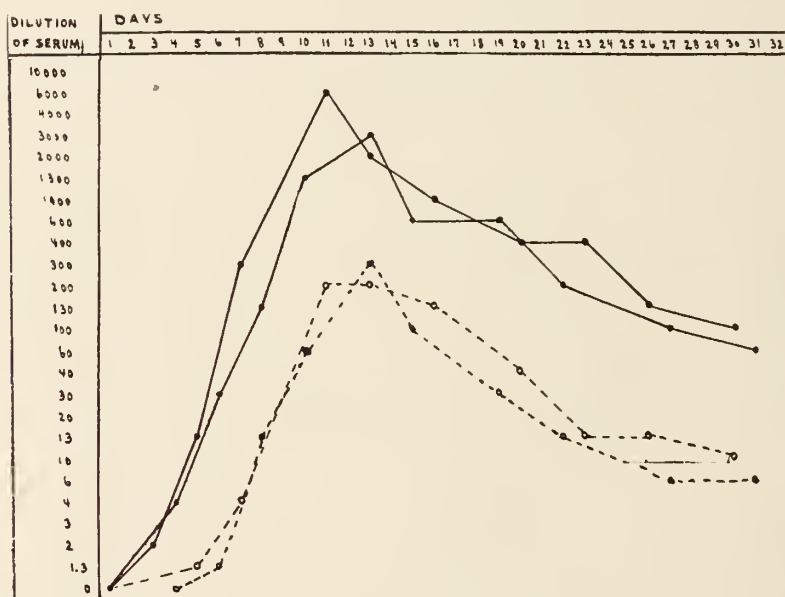


Chart 1.—The effects of sodium iodoxybenzoate on the production of specific hemolysin. Solid lines represent the specific lysin in blood of rabbits which on the first day received intravenous injection of 2.5 c.c. of washed ox corpuscles per kilogram of body weight, and one hour afterward 5 c.c. of 1 percent sodium iodoxybenzoate intravenously. The broken lines represent the specific lysin in blood of rabbits which on the first day received intravenous injection of 2.5 c.c. washed corpuscles per kilogram body weight.

benzoate could not be due to the small amount of oxygen which is added to the blood, but more likely to the stimulating or catalytic effect which this organic peroxid exerts on the oxidations in the tissues, the site of antibody formation. Further evidence that this substance acts on the tissues rather than on the blood is presented in the discussion of the influence of this substance on the local allergic reaction.

ACTION OF SODIUM IODOXYBENZOATE ON THE PRODUCTION OF AGGLUTININ

Rabbits of the same sex and species and practically the same weight and which had not been used for other work were injected with an old stock culture of the typhoid bacillus. Before the rabbits were used, the normal agglutinating power of the serum was determined.

Rabbits giving no agglutination of the typhoid bacillus in dilution of 1/20 before injection of typhoid bacilli were used for this work. Twenty-four-hour growths of a stock culture on standard agar slants were suspended in 0.85 percent sterile salt solution and killed by heating in a water bath at 60 C. for one hour. The rabbits received similar amounts of typhoid suspension, which in each case was equivalent to one twenty-four-hour slant agar growth, intraperitoneally. The control animal was given 5 c.c. of sterile salt solution intravenously, the other animal 5 c.c. of a sterile N/20 solution of sodium iodoxybenzoate intravenously, one hour after the injection of the typhoid bacilli. The animals were bled at intervals of two to four days and the agglutinating power of the serum determined. This was done by making dilutions of the serum as follows: 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1,280, and 1/2,560. All the dilutions were made up to 1 c.c., and 1 c.c. of the bacterial suspension, made from living, twenty-four-hour cultures in 0.85 percent salt solution, was added. The mixtures were incubated two hours at 37 C. and then examined at the end of twenty-four hours. Only complete agglutination was recorded.

TABLE 1

THE INFLUENCE OF SODIUM IODOXYBENZOATE ON THE PRODUCTION OF AGGLUTININ

Animal	Agglutinating Power of Serum							
	3 Days	4 Days	6 Days	9 Days	11 Days	14 Days	17 Days	20 Days
Control	1/20	1/20	1/40	1/160	1/640	1/320	1/160	1/80
Iodoxybenzoate	1/40	1/40	1/160	1/320	1/2560	1/1280		

The experiment demonstrates that sodium iodoxybenzoate has a marked stimulating effect on the production of specific agglutinin in rabbits immunized with killed typhoid bacilli. On the eleventh day, the agglutinating power of the rabbit injected with sodium iodoxybenzoate was four times as great as that of the control animal.

The experiment was repeated with another pair of animals with practically similar results, altho in this second experiment I used only 4 c.c. of N/20 sodium iodoxybenzoate intravenously.

TABLE 2

THE INFLUENCE OF SODIUM IODOXYBENZOATE ON THE PRODUCTION OF AGGLUTININ

Animal	Agglutinating Power of Serum							
	3 Days	5 Days	7 Days	9 Days	11 Days	14 Days	17 Days	20 Days
Control	1/20	1/20	1/40	1/40	1/320	1/160	1/160	1/80
Iodoxybenzoate	1/20	1/40	1/80	1/160	1/1280	1/640	1/320	1/160

The fact that sodium iodoxybenzoate stimulates the production of agglutinin as well as hemolysin indicates that oxidation plays a rôle in the production of both and that there must be a close relationship between oxidation and antibody formation. Whether the tissue acted upon in the production of both of these antibodies is the same or not cannot be determined by these experiments. As I have already stated, this action of sodium iodoxybenzoate cannot be due to any effect on the blood, for the small amount of oxygen which it contains would have little influence on oxidative processes in the blood stream.

Experiments, the results of which will be published later, indicate that sodium iodoxybenzoate also stimulates the production of opsonin in rabbits injected with staphylococci.

THE INFLUENCE OF SODIUM IODOXYBENZOATE ON THE TUBERCULIN REACTION

The influence of sodium iodoxybenzoate on the local allergic reaction was studied by Amberg and Knox,¹⁸ who found that this solution diminishes the intensity of the intracutaneous reaction when given intravenously in rabbits sensitized with horse serum. Iodbenzoic acid does not have this effect. These authors conclude that the solution does not influence the allergic reaction, but probably acts on the inflammatory processes because of a product of the allergic reaction. In a later study, Amberg¹⁹ has observed that sodium iodoxybenzoate has an inhibitory effect on the inflammatory reactions produced by irritating substances, such as mustard oil and diphtheria toxin when given intracutaneously. These results indicate that the action of sodium iodoxybenzoate is on the early stage of the inflammatory reaction produced by toxic substances formed at the site of injection.

That the mechanism of general anaphylaxis is not the same as that causing the local allergic reaction on intracutaneous injection of an allergen in a sensitized animal is suggested by the work of Meigs,²⁰ who concludes that the intracutaneous reaction is produced by the action of substances formed at the site of injection, which cause the inflammatory reaction.

Assuming that the tuberculin reaction is similar in the mechanism of its production to the other local allergic reactions and is therefore produced by the formation of toxic substances at the site of injection, we should expect sodium iodoxybenzoate to have a similar effect on this

18. Jour. Phar. and Exper. Therap., 1912, 3, p. 223.

19. Ztschr. f. ges. exper. Med., 1913, 2, p. 19.

20. Jour. Infect. Dis., 1914, 15, p. 541.

inflammatory reaction as on those studied by Amberg. The following results indicate that this is the case, for sodium iodoxybenzoate has a marked inhibitory effect on the local tuberculin reaction in tuberculous animals.

I have made a study of the influence of sodium iodoxybenzoate on the intracutaneous tuberculin reaction in some of the rabbits and guinea-pigs infected with well-developed, tuberculous lesions. The animals were placed at my disposal through the kindness of Dr. H. J. Corper. Those in each series of experiments had all been inoculated at the same time with similar amounts of bacilli. As controls were used tuberculous animals which were injected intracutaneously with 0.1 c.c. of human tuberculin, and this was immediately followed by the injection of salt solution, 0.85 percent. The control guinea-pigs were given the salt solution intraperitoneally; the rabbits intravenously. The treated animals were given similar quantities of the tuberculin and immediately afterward injected with neutral sodium iodoxybenzoate. The treated guinea-pigs received the drug intraperitoneally; the treated rabbits intravenously. The amounts of sodium iodoxybenzoate given to the treated animals and of salt solution given to the controls were 10 c.c. for the rabbits and 5 c.c. for the guinea-pigs. Measurements of the local reactions were made at the end of three, six, and twenty-four hours, by determining both the diameter and the thickness of the infiltration at the site of injection of the tuberculin. The measurements were made in millimeters with calipers.

The guinea-pigs were inoculated on November 27, 1912, with 0.02 mg. human tubercle bacilli in the inguinal region. On January 15, when intracutaneous tests were made, animal showed marked enlargement of local glands. Amount of tuberculin used was 0.1 c.c. of 1/5 human tuberculin intracutaneously in the shaved skin over abdomen. This was followed immediately by intraperitoneal injection of 5 c.c. of N/20 sodium iodoxybenzoate, and of 5 c.c. of 0.85 percent salt solution in control animal.

TABLE 3
EFFECT OF SODIUM IODOXYBENZOATE ON THE LOCAL TUBERCULIN REACTION IN GUINEA-PIGS
INOCULATED IN THE INGUINAL REGION

Guinea-pig Injected with Salt Solution			Guinea-pig Injected with Sodium Iodoxybenzoate		
Hours	Diameter	Thickness	Hours	Diameter	Thickness
3	20 mm.	2.3 mm.	3	0 mm.	1.3 mm.
6	30 mm.	2.7 mm.	6	10 mm.	1.5 mm.
24	28 mm.	2.4 mm.	24	12 mm.	2.0 mm.
Thickness of normal skin 1.3 mm.			Thickness of normal skin 1.2 mm.		
Guinea-pig Injected with Salt Solution			Guinea-pig Injected with Sodium Iodoxybenzoate		
Hours	Diameter	Thickness	Hours	Diameter	Thickness
3	24.0 mm.	2.1 mm.	3	0 mm.	1.4 mm.
6	28.0 mm.	2.4 mm.	6	8 mm.	1.5 mm.
24	33.0 mm.	3.4 mm.	24	18 mm.	1.5 mm.
Thickness of normal skin 1.1 mm.			Thickness of normal skin 1.2 mm.		

Two more series of experiments were carried out with very similar results. In all there was a marked difference between the animal

injected with sodium iodoxybenzoate and the control animal. The treated animals showed a marked diminution both in the diameter and thickness of the local area of inflammation. In two of the treated animals the reaction was so slight that it could be determined only by measuring the thickness of the skin fold at the site of injection.

The experiments were repeated in rabbits inoculated into the anterior chamber of the eye with human tubercle bacilli. In testing the influence of sodium iodoxybenzoate on the tuberculin reaction in these animals the drug was given by intravenous injections.

Rabbits were inoculated on November 15, 1912, with 0.02 mg. of human tubercle bacilli into the anterior chamber of the right eye. On January 24 when the intracutaneous tests were made, the animals showed distinct tubercle formation in the right eye. The length of time required for the tuberculin reaction to become positive following such an inoculation was determined. In an animal of this same series I found that the tuberculin test first became positive on January 7, seven weeks after the inoculation. One-tenth cubic centimeter of tuberculin was injected intracutaneously in the shaved skin over the abdomen. This was followed immediately by the injection of 10 c.c. of N/20 neutral sodium iodoxybenzoate intravenously in the treated animal and 10 c.c. of 0.85 percent salt solution in controls.

TABLE 4

EFFECT OF SODIUM IODOXYBENZOATE ON THE LOCAL TUBERCULIN REACTION IN RABBITS INOCULATED INTO THE ANTERIOR CHAMBER OF THE EYE

Rabbit Injected with Salt Solution			Rabbit Injected with Sodium Iodoxybenzoate		
Hours	Diameter	Thickness	Hours	Diameter	Thickness
3	10.0 mm.	1.6 mm.	3	0 mm.	1.4 mm.
6	18.0 mm.	2.0 mm.	6	10 mm.	1.6 mm.
24	30.0 mm.	3.5 mm.	24	15 mm.	2.0 mm.
Thickness of normal skin 1.3 mm.			Thickness of normal skin 1.4 mm.		

These results indicate that sodium iodoxybenzoate has a marked effect on the local tuberculin reaction in animals infected with tuberculosis. It diminishes the intensity of the reaction in guinea-pigs on intraperitoneal injection and also in rabbits on intravenous injection. If we assume that the local tuberculin reaction is a local inflammatory reaction produced as a result of the formation of toxic substances at the site of injection, which toxic substances are not produced in the normal animal, then the iodoxybenzoate may act by increasing the oxidative processes in the tissue in which the tuberculin is injected, so that the toxic substances are rendered less irritating because of a more rapid oxidation of the toxic substances. This would explain why the drug acts only in the early stages of the inflammatory reaction, also why repeated injections of iodoxybenzoate have no effect, as observed

by Amberg for inflammatory reactions produced by toxin. For, after the toxic substances have combined with the tissues, the action of the drug would be prevented, and substances, such as cyanids, which prevent or reduce normal oxidation by the tissues, would tend to increase local inflammatory reactions by interfering with oxidation of toxic substances. Whether, in the case of the tuberculin reaction, the drug acts by accelerating oxidation of the tuberculin itself or of the toxic substances produced by the action of antibody or enzyme present in the sensitized animal on the tuberculin remains to be determined.

In order to determine whether or not sodium iodoxybenzoate acts on tuberculin in vitro by oxidizing it I made the following experiment:

Five cubic centimeters of 1 percent sodium iodoxybenzoate solution was placed in each of two test-tubes. To one, 1 c.c. of the tuberculin used was added, to the other 1 c.c. of 0.85 percent salt solution. In a third test tube 5 c.c. of salt solution and 1 c.c. of tuberculin were placed. The tubes were incubated at 37 C. for twenty-four hours. At the end of this time I determined the amount of oxygen present in each tube by the method which I use for determining the amount of available oxygen in the drug, i. e., by the liberation of I from KI in acid solution and titrating the iodine with N/10 sodium thiosulphate.

	N/10 Thio Required
Iodoxybenzoate 1 percent 5 c.c. + salt solution 1 c.c.	7.0 c.c.
Iodoxybenzoate 1 percent 5 c.c. + tuberculin 1 c.c.	5.2 c.c.
Salt solution 5 c.c. + tuberculin 1 c.c.	0.0 c.c.

These results show that tuberculin is oxidized by sodium iodoxybenzoate in vitro. In fact, 1 c.c. of the tuberculin combined with 25 percent of the available oxygen in 5 c.c. of 1 percent iodoxybenzoate. Calculating the amount of oxygen, I find that 1 c.c. of tuberculin combined with 1.44 mg. of oxygen (1 c.c. of N/10 thio corresponds to 0.0008 gm. of oxygen). Whether or not tuberculin treated with iodoxybenzoate in vitro produces a less marked local reaction or loses its power of producing the local inflammatory reaction in tuberculous animals as a result of its oxidation remains to be determined. Experiments now under way indicate that tuberculin is distinctly less toxic after its oxidation by certain agents. These results suggest the possibility of producing a tuberculin which has been deprived of its toxicity or whose toxicity has been reduced by the action of certain oxidizing agents. Such tuberculin might be of value in therapeutics if it does not lose its curative effect when its toxic action is reduced.

SUMMARY AND CONCLUSIONS

In previous publications I have demonstrated a relationship between oxidation and the process of phagocytosis, finding that substances

which stimulate oxidations *in vivo* have a stimulating effect on phagocytosis, whereas substances which depress oxidations have an inhibitory effect on phagocytosis *in vivo* as well as *in vitro*. In order to study the influence of increased oxidation on other immune reactions, I have used sodium iodoxybenzoate, an organic peroxid, which has a marked effect on oxidative processes in the body. These properties are attributable to the oxygen combined with the iodine in the molecule. Sodium iodoxybenzoate has little effect on the blood and can therefore be injected intravenously. It has a stimulating effect on phagocytosis *in vitro* and possesses distinct germicidal properties.

Sodium iodoxybenzoate stimulates the production of hemolysin and agglutinin in rabbits when injected intravenously shortly after immunization. The results show that there is a close relationship between the production of antibodies and the oxidative processes. The substance probably acts by accelerating oxidations in the tissues which are the site of antibody formation. This acceleration is produced probably by some catalytic effect of the sodium iodoxybenzoate, for the small amount of oxygen injected into the circulation could not have this effect. That the action of this compound is exerted on the tissues is shown by the fact that the substance diminishes the intensity of the local allergic reaction in sensitized animals. It has a marked inhibitory action on the local tuberculin reaction in tuberculous animals. This action is due to a more active oxidation by the tissues of the toxic substances which cause the inflammatory reaction. Whether these toxic substances acted upon are the ones present in the tuberculin or are produced at the site of the inflammatory reaction in the tuberculous animal has not been determined. However, sodium iodoxybenzoate has the power of oxidizing tuberculin *in vitro*, and this oxidation is accompanied by a decrease in the toxicity when injected into normal animals.

All these results suggest that there is a close relationship between oxidation and immune processes, for a substance which is an organic peroxid stimulates the production of antibodies. It probably sets up in the tissues, which are the site of antibody formation, a more active oxidation, not the result of the amount of oxygen which it contains but because of some catalytic effect. That the compound can influence tissue oxidation is shown by its inhibitory action on the local allergic reaction, a reaction due, in part at least, to interference with oxidation at the site of the reaction.

SIMULTANEOUS INFECTION IN A CHILD WITH TUBERCLE BACILLI OF THE HUMAN AND OF THE BOVINE TYPE *

ARENT DE BESCHE

(From the Pathological-Anatomical Institute in Christiania, Norway)

In 1913 I published a series of investigations concerning tuberculous infections in childhood.¹ This work constitutes in certain respects a continuation of similar investigations by Harbitz,² who has found with respect to tuberculosis in children up to fifteen years of age that 286 cases, or 59 percent, were free from tuberculous infection, while 198, or 41 percent, were infected. Among these 119, or 60 percent, died from tuberculosis; and 52, or 26.3 percent, had latent tuberculosis, and 27, or 13.2 percent, latent tubercle bacilli.

The most important results of my work so far may be summarized as follows: On injection into guinea-pigs of lymph glands obtained post mortem from 134 unselected cases, there resulted tuberculous infection in 52, or 38.8 percent, of the cases. In each individual case there were injected cervical and mesenteric lymph glands, in some cases also the bronchial glands. The percentage of tuberculous infection was lowest among children less than a year old and highest among the oldest.

According to age, the children investigated may be grouped as follows:

Age	Number of Cases Examined	Number of Cases Infected with Tuberculosis
Under one year.....	58	14—24.2 percent
One to three years.....	28	11—39.3 percent
Three to five years.....	14	7—50.0 percent
Five to fifteen years.....	34	20—58.8 percent
Total	134	52—38.0 percent

In twenty-eight of the cases infected with tuberculosis, the cause of death was tuberculosis. In fourteen cases, tuberculosis (latent form)

* Received for publication February 10, 1915.

1. Norsk. Mag. f. Lægevidensk, 1913, 74, Supplement; reviewed in Deutsch. Med. Wehnschr., 1913, 39, p. 452.

2. Jour. Infect. Dis., 1905, 2, p. 143; Norsk Mag. f. Lægevidensk., 1913, 74, p. 1.

was found as an incident at the post mortem; and in ten cases, tubercle bacilli were present in the lymph glands, in which morbid changes could not be demonstrated macroscopically or microscopically. In the majority of the cases, there seemed to be a general infection of the lymph glands, since there resulted a tuberculous infection from both groups of glands in 70.4 percent of the cases in which both groups were injected successfully into guinea-pigs.

Fifty of the cases of tuberculous children have been studied bacteriologically, tuberculous material being injected into guinea-pigs, and tubercle bacilli isolated in pure culture from the infected animals. The growth of the bacilli was then studied on glycerin broth, their virulence being tested by injection into rabbits, using at least two rabbits in each case; in several cases, calves were also injected.

In forty-six cases, tubercle bacilli of the human type were found; in three cases, of the bovine type; in one case, there was isolated a type of bacillus that was difficult to incorporate into either of the two main groups. This strain we had to regard at first as "atypical."

As will be observed from this brief résumé it is possible, in the great majority of the strains of tubercle bacilli that are isolated in cases of tuberculosis in children, to tell whether they should be classed under the human or the bovine type; it is seldom one encounters a strain that must be classed as atypical, in my work only one instance occurring.

As is well known there has existed a marked difference of opinion as to how these atypical strains are to be regarded. A number of investigators have declared that in the strains that reveal atypical characteristics, we have transition forms between the human and bovine types, in other words, the actual proof of the transformation in the human body of the bovine to the human type of tubercle bacillus. It has been stated, particularly by the English Tuberculosis Commission in their Final Report, that strains which appear as transitional forms cannot be regarded as such, because a closer study of the cultures may show that they are strains in which the bovine and human tubercle bacilli are mixed ("mixed virus").

Since the strain I have studied has been found to consist of both bovine and human bacilli, I wish to report the case with the results of the work connected therewith.

The culture was isolated from a mesenteric gland of a child, eight months old, who died of general tuberculosis. On isolation in the

usual manner the bacilli from this case showed a rapid and luxuriant growth in glycerin broth and also proved to be acid-producing (Theobald Smith's reaction). In other words, the bacilli showed the cultural peculiarities characteristic of tubercle bacilli of the human type. Morphologically, the bacilli were long, slender, and beaded, such as one recognizes in the human type. The virulence of the bacillus is evident from the following experiment:

On May 12, 1910, a rabbit was injected subcutaneously over the abdomen with 0.01 gr. culture-substance from broth. On October 23, the rabbit was killed. It was greatly emaciated, the weight when injected being 2,750 gm.; when killed, 1,500 gm. At the site of injection was an abscess, as large as a hen's egg, filled with a large amount of thick, yellowish-white pus. In the inguinal and axillary lymph glands were a few tubercles. In the lungs were numerous tubercles of a gray and yellowish color, closely massed together, and reaching the size of a hempseed; there were also a few pea-sized nodules.

On April 22, a rabbit was injected intravenously with 0.001 gr. of the culture substance. After about five weeks the rabbit died. Both lungs showed a complete infiltration with yellowish, confluent tubercles; otherwise, no tuberculosis was found in the rabbit.

On November 8, 1911, a calf was injected subcutaneously in the neck with 0.05 gr. of the culture substance of a growing broth culture. The calf, greatly emaciated, was killed three months afterward. At the site of injection was an abscess, the size of a child's head, filled with a yellowish pus in which tubercle bacilli were demonstrated in large numbers. On the neck and in the chest cavities, were numerous tuberculous glands, some as large as a walnut, and in both lungs were miliary tubercles and nodules the size of a pea.

As is evident from these results, we are dealing with a strain of tubercle bacillus which reveals morphologically and culturally the characteristics of the human type. On the other hand, the virulence test in animals indicates a virulence for animals not corresponding to the human type, the resulting tuberculosis in the rabbit and calf being too generalized. This strain therefore can not be unconditionally classified as either of human or bovine type, but must be designated as "atypical."

In order to study the strain more closely the following experiment was made:

Cultures were made from the abscess of the neck of the calf and also from the cervical lymph glands. Altogether twenty tubes of glycerin potato and ten tubes of glycerin serum were inoculated. After six weeks there appeared a scanty growth on three tubes of glycerin potato. Subcultures were made and finally, after several transfers, I succeeded in obtaining a surface growth on glycerin broth, the bacilli growing as a fine film on the surface, with only a few denser areas here and there. From a growing broth culture 0.001 gr. of culture substance was injected into a rabbit intravenously. The rabbit became

greatly emaciated and died about three weeks after the injection, showing a great many densely packed tubercles in both lungs, together with a few tubercles scattered through one kidney.

It was therefore plain that the tubercle bacilli isolated from the tuberculous calf possessed the marked characteristics of tubercle bacilli of the bovine type, hence bovine tubercle bacilli must therefore have been present in the original culture.

Now in order to demonstrate whether bacilli of the human type were present in the original culture, I made a series of plate cultures, to obtain, if possible, isolated colonies of the human type. Making use of large, flat slices of glycerin potato, I succeeded on these in obtaining isolated colonies which could be used for my purpose. For the further study of the colonies, I selected the colonies that first grew, with the idea that from these I had the best prospect of obtaining the human type in pure culture, since this bacillus grows more rapidly and more easily on artificial media than the bovine bacillus.

From a plate culture with isolated colonies of bacilli, I selected six colonies and transplanted them on ordinary slanted glycerin potato media and after growth appeared I transferred the cultures to glycerin broth. All of these cultures proved to grow in a manner characteristic of the human type, producing a luxuriant growth on glycerin broth. In order to test the virulence, 0.01 gr. of the bacterial substance from each of the broth cultures was injected subcutaneously into rabbits, each of the cultures being tested with two rabbits. The results of these injections are given in Table 1.

It may be concluded from the table that there are three colonies (1, 2, and 5) in which the bacillus shows itself to be avirulent, or slightly virulent, toward rabbits, there being only unimportant lesions at the site of injection. Therefore these bacilli, which were isolated from the original culture, must be considered as of the human type. Of the remaining colonies, Colony 6 also showed itself to contain bacilli of slight virulence toward rabbits and yet toward one of the rabbits it showed virulence so severe that one cannot conclude with full assurance that this colony was pure, that is, that it consisted only of bacilli of the human type. As regards the other colonies, we may assume from the result of injections into rabbits, the injection of 0.01 gr. of culture substance having caused lesions of a greater or less degree in the internal organs, that the two types of bacilli were present together in these colonies.

TABLE 1

RESULTS OF INJECTION OF TUBERCULOUS BACTERIAL SUBSTANCE INTO RABBITS

Colony	Rabbits Injected Subcutaneously with 0.01 Gr. of Bacterial Substance	Date of Injection	Date Killed	Results of Autopsy
1	Rabbit 1. Weight, 2,100 gr....	5/24/13	9/ 3/13	Weight, 2,450 gr. At site of injection pea-sized nodule, partially caseated; no tuberculous changes of internal organs.
	Rabbit 2. Weight, 2,300 gr....	5/24/13	9/ 3/13	Weight, 2,220 gr. No lesion at site of injection; no changes in internal organs.
2	Rabbit 1. Weight, 2,210 gr....	5/24/13	9/ 5/13	Weight, 2,500 gr. A gray fibrous scar at site of inoculation; no tuberculous changes.
	Rabbit 2. Weight, 2,100 gr....	5/24/13	9/ 5/13	Weight, 1,950 gr. A pea-sized abscess at site of injection; no tuberculous changes of the internal organs.
3	Rabbit 1. Weight, 2,200 gr....	5/24/13	9/ 5/13	Weight, 1,700 gr. Numerous gray and yellow tubercles in both lungs; single tubercles in liver and spleen, none in kidneys; abscess of walnut size at site of injection; tuberculosis of regional lymph glands.
	Rabbit 2.....	5/24/13	9/ 5/13	Weight, 1,810 gr. Numerous gray, and a few yellow tubercles in the lungs, otherwise no tuberculosis of organs; small abscess at site of injection; tuberculosis of regional lymph glands.
4	Rabbit 1. Weight, 2,650 gr....	5/26/13	9/ 8/13	Weight, 2,320 gr. Single gray tubercles in lungs and spleen; walnut-sized callous abscess at site of inoculation; no tuberculosis of regional lymph glands.
	Rabbit 2. Weight, 2,480 gr....	5/26/13	9/ 8/13	Weight, 2,020 gr. In the right lung numerous, in the left few, gray and yellow tubercles; abscess scarcely of pea-size at site of injection; tubercles in regional lymph glands.
5	Rabbit 1. Weight, 2,300 gr....	5/26/13	9/12/13	Weight, 2,800 gr. Pea-sized caseous nodule at site of injection; no tuberculosis of organs.
	Rabbit 2. Weight, 2,180 gr....	5/26/13	12/ 9/13	Weight, 2,400 gr. No lesion at site of inoculation; no tuberculosis of internal organs.
6	Rabbit 1. Weight, 2,560 gr....	5/26/13	9/12/13	Weight, 2,800 gr. Millet-seed-sized nodule at site of inoculation; no tuberculosis of internal organs.
	Rabbit 2.....	5/26/13	9/12/13	Weight, 2,250 gr. Caseous nodule at site of injection; single tubercles in regional lymph glands; a few tubercles in both lungs.

CONCLUSION

The strain of tubercle bacilli isolated from the mesenteric gland of a tuberculous child which on first examination had to be classified as "atypical," upon closer study proved to be a "mixed virus," since both types of tubercle bacilli were isolated from the cultures. The individual from whom the cultures were isolated had been infected with tubercle bacilli both of the human and of the bovine type.

THE ETIOLOGY AND EXPERIMENTAL PRODUCTION OF ERYTHEMA NODOSUM *

EDWARD C. ROSENOW

(From the Memorial Institute for Infectious Diseases, Chicago)

WITH PLATES 17 TO 22

Erythema nodosum occasionally attacks several members of the same family¹ and may occur in epidemic form.² Osler³ especially has emphasized its close relation to rheumatism and endocarditis, and Brian⁴ has pointed out that it not infrequently develops after tonsillitis. These are some of the indications that erythema nodosum is a specific infectious disease, but as yet no one appears to have demonstrated the same microorganism in the nodes in a series of cases, or produced the disease in animals. In this paper I wish to record briefly a series of cases in which a bacteriological study of the blood, of the probable infection atrium, and of excised nodes was made with almost uniformly positive results, lesions quite like those of erythema nodosum developing in animals on intravenous injection of the organisms isolated.

TECHNIC

The nodes were excised after thoroughly sterilizing the skin, usually with tincture of iodine. In some instances the excision was made by one linear incision after dissecting the skin from the infiltrated node; in other instances the skin and node were removed by an elliptical incision. The excised tissue was covered at once with sterile gauze and taken to the laboratory. Approximately one-half of the material removed was fixed in Zenker's solution or absolute alcohol, the rest being emulsified, after surface sterilization, in broth or salt solution in a mortar in a specially devised, sterile air chamber. In those cases in which the skin was also excised separate cultures were made of the node and the skin. Shake cultures of the emulsion were made in tall columns (10-12 cm.) of dextrose broth, ascites dextrose broth, and on blood agar and Loeffler's serum slants. One slant of each was incubated under anaerobic conditions. The tubes were incubated and examined daily for at least ten days before being discarded. The individual colonies were "fished" from the top when not too deeply situated, and obtained from the deeper layers by breaking the tube at the proper level by means of a glass cutter and red

* Received for publication February 10, 1915.

1. Knipe: Brit. Med. Jour., 1882, 2, p. 974; Demme: Fortschr. d. Med., 1888, 7, p. 241; Brömmer: Norsk Mag. f. Lægevidensk., 1906, 7, p. 34.
2. Eichhorst: Spec. Path. u. Therap., 1907, 3, p. 609.
3. Am. Jour. Med. Sc., 1904, 127, p. 1.
4. Deutsch. Arch. f. klin. Med., 1911, 104, p. 272.

hot glass bead at the end of a pipette. One or more tubes of ascites dextrose agar were always incubated as controls. Cultures from the supposed infection atrium were made in the same way. Blood was laked in distilled water after pipetting off most of the serum from the citrated blood. The serum and the sediment of the laked portion were planted into tall tubes of ascites dextrose agar in the usual way.

The animals while being chloroformed were held in the hands so as to prevent injury to the subcutaneous structures. Owing to the hair it was not usually possible to determine the presence of subcutaneous lesions before death. They were searched for in the deeper layers as the skin was removed. The animals were examined as soon after death as possible.

The portion of the node put aside for microscopic study was fixed in absolute alcohol, or Zenker's fluid, imbedded in paraffin, and stained with hematoxylin and eosin. Owing to the fact that the bacilli do not stain by the less penetrating stains and lose their stain readily when treated by Gram's method, the demonstration of bacilli in the tissues is difficult. Various staining methods were tried without success, but by decolorizing only partially to a pale but distinct blue in the Gram-Weigert method bacilli have been found in varying number in all the cases.

CASES

CASE 808.—A young woman in the service of Dr. Sippy at the Presbyterian Hospital. She came to the hospital complaining of general ill health with rather vague pains in legs and arms, which were never severe enough to keep her awake at night; subject to headaches and backache; constipated; no sore throat; appetite poor.

On November 26, 1913, patient noticed two small, reddened, raised, and tender nodules in palm of the hand and soon after some on the knees and on the anterior aspect of the legs. On November 30, a moderate number of tender, subcutaneous, erythematous nodes appeared chiefly over the legs and forearms. This was associated with a mild tonsillitis, definite enlargement of the cervical lymph glands, and marked tenderness in the muscles of the neck. Previous to the attack of erythema nodosum the temperature was practically normal, occasionally going to 99.6 F., at one time to 101 F. After the appearance of the nodes the temperature ranged from 99 to 102 F. for nearly two weeks and then gradually came down to normal, there being a slight rise the day after the administration of a small dose of a vaccine prepared from the organism isolated from a node and from the cervical lymph gland. The leukocytosis on November 28 was 16,000, on November 24, 13,400, hemoglobin 86 percent. Repeated examinations of the stools were negative. The urine was normal except on December 4, when there was present a small amount of serum and nucleo-albumin.

On December 4, a navy-bean-sized lymph gland from the anterior margin of the left trapezius muscle and a portion of a subcutaneous, circumscribed, infiltrated, red area from the right forearm were removed under strict aseptic precautions by Dr. Lewis. Cultures were made at once. On December 7, forty very fine colonies in ascites dextrose agar developed from the subcutaneous node and fifteen from the lymph gland; also a smaller number of larger, more opaque, spherical colonies. Smears from the former show moderately gram-positive, non-acid fast, polymorphic, often beaded, and sometimes clubbed, bacilli. Smears from the larger colonies show small, short, gram-positive bacilli. Smears from the water of condensation of Loeffler's serum slants show the same bacillus in pure form. Sub-cultures on blood agar plates of the bacilli

gave small, moist, non-adherent, non-hemolysing colonies with a distinct yellowish-green color on transmitted light.

CASE 904.—A woman, 18 years of age, in Dr. Billings' service at the Presbyterian Hospital. On entrance she complained of enlarged, swollen, painful joints with deformities, crepitus and limitation of motion, with muscular contractures and muscle tenderness; and a considerable loss of weight. She had been subject to repeated attacks of tonsillitis. The present trouble had begun two and one-half years before with tenderness in the soles of the feet. Knees and ankles soon became swollen, leg muscles became sore with a gradual extension, and other joints and muscles became involved in the following order: hands, ankles, shoulders, elbows, wrists, spine. The patient was fairly well nourished, well developed; her frame small, bony; her movements difficult because of stiffness and pain in joints; the superficial lymph glands easily palpable. There were many carious teeth; one alveolar abscess opposite the second left lower molar; the pharynx red; tonsils large with deep crypts from which cheesy exudate was expressed. The thyroid was not enlarged and the heart tones were clear.

On February 6, 1913, tonsillectomy was followed by slight increase in temperature for a day or two. On February 16, a number of teeth were extracted, followed on the next day by fever from 102 to 105 F. for nearly three weeks, associated with pericarditis, pleurisy with effusion, bronchopneumonia, exacerbation of the joint sensitiveness, and successive crops of erythematous nodes of the skin chiefly over the forearms and legs, acute dilatation with acute multiple ulceration of the stomach shortly before death, February 28.

On February 18, cultures were made from the excised erythematous node, from the alveolar abscess, the blood, and the pleural fluid. On February 21, cultures from the subcutaneous node showed four colonies of a short, markedly polymorphic bacillus. The skin overlying the node showed one colony of staphylococcus and one colony of the bacillus. The blood showed *B. welchii* and the diphtheroid bacillus; the pleural fluid, the diphtheroid bacillus only; and the material from the alveolar abscess, the bacillus and streptococci. This bacillus was isolated also in moderate numbers after death from small, firm vegetations on the mitral valve and from the ulcer in the mucous membrane of the stomach.

Sections of the node showed hemorrhages, round cell and leukocytic infiltration, and a few gram-staining bacilli in the layers immediately beneath the cutis.

CASE 906.—A 9-year-old girl in the service of Dr. Hess in the Cook County Hospital. An attack of tonsillitis was followed in two weeks by general joint pains with definite swelling and redness only of the left elbow joint. As the joint symptoms disappeared, there developed without a chill a fever of 103 F. and a crop of red, tender, painful, circumscribed subcutaneous nodes, 0.5 to 2 cm., chiefly over the anterior aspect of the legs and forearms (Fig. 2). Patient did not now (two days later) complain of sore throat. She was well nourished and well developed. The tonsils were red and enlarged, crypts filled with cheesy exudate, small superficial ulcers on the right anterior pillar; cervical lymph glands palpable. Recovery uneventful.

Cultures were made from the tonsillar crypt, the ulcer in the throat, the blood, and from an excised node in the anterior aspect of the leg. Two days later the cultures from tonsils and ulcer, on blood agar plates, showed slightly hemolytic, and a few green-producing, streptococci with a few smaller, grayish-brown colonies which did not affect the medium and which showed bacilli similar to those isolated from the blood and node in pure culture. From the blood there developed four, and from the node ten, colonies, of a small, usually short,

sometimes clubbed, gram-positive, non-acid fast, non-motile diplobacillus. All gradations between cocci, diplococci, and distinct bacilli could be made out in smears from each of the colonies. Those nearer the top of the tube showed relatively more coccus forms, those deeper down more bacillary forms.

Rabbit 619.—Injected intravenously on March 6 with the growth from three Loeffler's blood serum slants and five days later with 5 c.c. of a twenty-four-hour anaerobic dextrose broth culture.

March 14, chloroformed and examined at once: Marked subcutaneous hemorrhage around place of injection; fading yellowish-brown edematous area over the inner aspect of thighs, the subcutaneous glands draining these areas being large and hyperemic; no other gross lesions. Blood, subcutaneous areas, and glands yielded streptococci and bacillary forms similar to those in the patient.

Rabbit 620.—Injected intravenously on March 7 with 5 c.c. of an anaerobic culture in ascites dextrose broth with sterile tissue.

March 9, seemed well; no arthritis; marked redness around place of injection.

March 10, found dead: Three subcutaneous, erythematous areas (0.5 to 1.5 cm.), one on outer aspect of right thigh, one over the anterior aspect of the chest wall, the other at the base of the left ear. The area in each instance was situated around easily visible blood vessels. The subcutaneous lymph glands draining two of these areas were large and hyperemic; no enlargement of lymph glands otherwise. There were no other gross lesions except localized, whitish streaks in the medulla, and small, embolic, white areas in the cortex, of the kidneys. Cultures from the blood gave grayish-green colonies on blood agar plates. Smears showed mostly coccoid forms, sometimes arranged in short chains, but also as distinct bacilli. Subcultures of these in tissue broth showed distinct chains of from four to twelve members, together with bacillary forms. Cultures from the nodes and glands yielded similar colonies, but the bacillary forms predominated in numbers. The subcultures of the coccoid forms in dextrose broth showed streptococci, diplococci, many single cocci, often arranged in groups.

Rabbit 623.—Injected intravenously on March 11 with 7 c.c. of a forty-eight-hour dextrose broth culture containing sterile tissue.

March 20, seemed well; chloroformed: Fading areas of hemorrhage in the skin over the back, shoulders, and legs, and enlarged lymph glands in the axilla and groin; healing subendothelial nodule in the tricuspid valve; no other lesions. Cultures negative.

Dog 72.—Injected intravenously on March 11 with the growth from 60 c.c. ascites dextrose broth. No immediate symptoms. March 14, the injection was repeated with 15 c.c. of tissue broth culture.

March 17, seemed well; chloroformed: Five distinct, circumscribed, red, erythematous, and two hemorrhagic, areas in the more superficial layers of the skin. The largest area measured 2 by 5 cm., the next largest, over the posterior portion of the back, 2 by 3 cm., and the smallest area, 0.5 by 1 cm., over the anterior aspect of the left hind leg. A large number of small, fading hemorrhagic areas in the more superficial layers of the skin. Subcutaneous lymph glands draining two of the more edematous areas large and hyperemic. The areas usually surrounded easily visible blood vessels. A hemorrhagic area in the right eye near the limbus at the point of the insertion of the internal rectus muscle. Stomach, intestines, heart, lungs, kidneys, bladder, adrenals, thyroid, pancreas, liver, testicles, muscles, and joints showed no changes. A moderate

number of small meningeal hemorrhages. Cultures from the blood, joints, and bile, sterile. Subcutaneous areas and glands yielded both non-hemolysing and hemolysing streptococci and diphtheroid bacilli.

March 31, a stab from one of the colonies showing bacilli on blood agar, into ascites dextrose agar, containing sterile tissue in the bottom, gave cocci resembling staphylococci and diplococci at the top of the tube; gram-positive, often long, straight bacilli at the bottom near the piece of tissue, where growth is very scant; and clubbed, barred bacilli, elongated diplococci, and round cocci in the middle portion.

The affinity of this organism for the skin is shown in Table 1.

Rabbit 633.—Injected intravenously on March 22 with 6 c.c. of a twenty-four-hour culture of this strain after passage through Rabbit 629 and Rabbit 619 (Table 1). Blood agar plate cultures of the bacteria injected showed small, grayish, non-hemolysing colonies only.

March 24, seemed quite well; chloroformed: A few small, brownish, discolored hemorrhagic areas in the skin of the lower part of the thighs, and four similar areas over the front legs; six hemorrhages in capsule of liver; fluid from the knee joints turbid. Cultures from the blood and joints in dextrose broth showed short, chain-producing streptococci. Blood agar plates showed a few hemolysing, and a moderate number of non-hemolysing, gray colonies of streptococci both from the blood and joint fluid.

Rabbit 634.—Injected intravenously on March 22 with 6 c.c. of tissue broth culture of the same strain as that of streptococcus isolated from the blood of Rabbit 627. Blood agar plates of the culture injected showed small, grayish-green, non-hemolysing colonies only.

March 24, chloroformed: Four subcutaneous hemorrhages and three in the intercostal muscles and fascia; joint fluids distinctly turbid. Cultures from the blood and joints in broth yielded short, chained streptococci. On blood agar plates, blood gave gray, non-hemolysing colonies only, while the joint fluid showed these, and three hemolysing colonies as well.

Rabbit 640.—Injected intravenously on March 25 with 10 c.c. of a broth culture of the strain from the blood of Rabbit 633.

March 27, found dead: No subcutaneous hemorrhages; a few small hemorrhages in the lungs, and in the more tendinous portion of the muscles; blood markedly hemolysed; joint fluid markedly turbid; no other gross lesions. Cultures from the blood and joints showed a large number of hemolysing streptococci.

Rabbit 642.—Injected intravenously on March 28 with 4 c.c. of the broth culture of the hemolytic streptococcus isolated from the blood of Rabbit 640.

March 31, found dead: Serofibrinous pericarditis, inflammation of fascia over the left thorax, and multiple suppurative arthritis; fluid from right knee joint turbid, that from the shoulder and left knee joint less turbid, while that from the intervertebral joints was clear; no hemorrhages in the skin; kidneys showed whitish, elongated areas in the medulla and hemorrhagic infarct; no other lesions. Cultures from shoulder and knee joints and from pericardium gave a large number of hemolysing streptococci, while those from the blood gave a small number.

CASE 911.—Girl, 17 years of age, in the service of Dr. Billings' at the Presbyterian Hospital. She was suffering with malaise, severe headache, chills and fever with a dry cough, substernal pain, and dyspnea on exertion. After six days, red, tender nodules appeared under the skin on the front of the leg and

the forearm, and pain developed in the muscles of the left side of the neck and shoulder. She had had an attack of severe pain in the shoulders and neck two months before. Nutrition and development were normal; teeth decayed but gums normal; previous tonsillectomy; numerous circumscribed, red, brown, and purple, tender nodes (0.5 to 4 cm.) over lower third of thighs and legs and over both forearms with some degree of symmetry. Pericarditis with effusion was found. The thyroid gland gradually enlarged and there was developed distinct tremor of the fingers when in extension. There was no distinct swelling of joints, but tenderness of the muscles of the neck and pain in moving the left shoulder. The temperature ranged between 101 and 103 F. for ten days and then dropped to normal. Blood was normal except for a leukocytosis of 13,600. The urine gave a small amount of albumin and a moderate number of leukocytes. Two blood cultures negative.

An infiltrated node over the anterior middle portion of the thigh was excised with a small piece of skin, which was removed, cultures being made separately from the node and the skin. Five colonies of a moderately polymorphic, diphtheroid bacillus in pure culture developed from the node in ascites dextrose agar; the same bacillus grew also in the water of condensation on Loeffler's blood serum slant. Cultures from the skin gave three colonies of staphylococcus albus. Subcultures from one of the colonies in dextrose agar into dextrose broth, both with and without tissue, and on Loeffler's serum and blood agar slants, all gave rather long, often barred and clubbed, gram-positive bacilli, resembling pseudo-diphtheria bacilli. Blood agar plates from tissue broth gave rather brownish-green, moist colonies of long, barred and clubbed bacilli. Stabs into tall tubes of ascites dextrose agar with a piece of sterile tissue at the bottom gave large, clubbed bacilli at the bottom, and short, thick, granular bacilli at the top.

Guinea-Pig 1183.—Injected intravenously on March 20, with 7 c.c. of an eight-day tissue broth culture.

March 21, chloroformed: Hemorrhages in deep layers of the skin over the abdomen and in the skin over both hind legs, in each instance situated along the course of easily visible blood vessels. Cultures from the blood on blood agar plates gave ten hemolysing colonies of streptococci.

Rabbit 630.—Injected intravenously on March 20 with the growth from one blood agar slant.

March 22, seemed quite well; chloroformed: Three small subcutaneous hemorrhages, one over the shoulder, the others over the hind extremities; one hemorrhage in the tendinous portion of the flexor muscles of the leg. Cultures negative.

Guinea-pig 1189.—Injected subcutaneously and intraperitoneally on March 23 with 10 c.c. of a tissue broth culture of the organism isolated from Guinea-pig 1183.

March 24, found dead: No lesions of the skin, except rather marked infiltration at the point of injection and a mild peritonitis. Cultures from the peritoneum gave large numbers, from the blood small numbers, of grayish, non-hemolysing, and a few moderately hemolysing, colonies of streptococci. Smears from some of the non-hemolysing colonies showed mostly cocci but also a few bacillus forms, while the hemolysing colonies showed streptococci only.

Rabbit 641.—Injected intravenously on March 26 with 10 c.c. of a twenty-four-hour tissue broth culture of the strain isolated from Guinea-pig 1189. March 28, found dead: Six small erythematous areas in deeper layers of the

skin situated along visible blood vessels; multiple arthritis; no other lesions. Cultures from the blood and joints gave a moderate number of markedly hemolysing streptococci.

CASE 929.—Girl, 18 years of age, in service of Dr. Tice and Dr. Slaymaker, Cook County Hospital. Illness had begun one week before with pain and swelling in the legs, associated with erythematous, raised, patchy, and tender eruptions of the skin over the front of the legs; ankles and knee joints painful and stiff, but not swollen; no sore throat, no chill, but malaise and fever for some days. There was no history of rheumatism. The patient was well nourished, well developed, in good general health; teeth in fair condition; tonsils slightly enlarged; skin over the tibia presented red, raised, tender, dime-sized edematous patches. One node just below the left knee was hard, shot-like, and movable under the skin. There was a slight tenderness over the ankle and knee joints but no swelling or pain on walking. Leukocytes 11,000. Urine contained trace of albumin, a few reds, but no casts. Patient was placed on large doses of sodium salicylate and sodium bicarbonate, but new nodes developed in successive crops over the skin of the legs and forearms at intervals of from seven to ten days for five weeks. Recovery.

March 19, cultures were made from an excised subcutaneous tender node on the forearm which had appeared three days before.

March 22, approximately 1,800 colonies had developed in shake cultures and growths on all the other media of a peculiar gram-staining, non-acid fast, non-motile, small, short, polymorphic bacillus (Figs. 3 and 4).

Guinea-Pig 1184.—Injected intravenously on March 22 with 2 c.c. of a twenty-four-hour culture in ascites dextrose broth containing sterile tissue, the organism appearing as a bacillus.

March 24, seemed quite well; chloroformed: Large number of small, subcutaneous, fading hemorrhages, most numerous over the back, most of these areas showing a distinct relation to the blood vessels; one larger hemorrhage with distinct infiltration and edema over the abdomen; one large hemorrhage under the visceral pleura; no other lesions. Cultures in broth from the blood gave streptococci producing rather large, long chains, and no bacillus forms.

Guinea-Pig 1187.—Injected intravenously on March 23 with the growth from 20 c.c. of ascites dextrose broth, the organism appearing more as a non-hemolytic streptococcus.

March 24, found dead: Moderate number of small hemorrhages in the deeper layers of the skin and flat muscles of the back and thorax; two symmetrically placed hemorrhages in the fascia and muscles of the legs; no other lesions. Cultures from the blood gave moderate number of grayish, non-hemolysing colonies and some producing a narrow, hazy zone of hemolysis.

Dog 75.—Injected intravenously on March 23 with growth from 150 c.c. of ascites dextrose broth, the organism appearing as a non-hemolysing streptococcus.

Death two hours after injection with vomiting and purging: Numerous small subcutaneous and cutaneous hemorrhages; five larger hemorrhages situated over the back and shoulder, the latter symmetrical; three hemorrhages in the flat, more tendinous, portions of muscles; two situated symmetrically in the flat muscles of the scapula; mucous membrane of the stomach and intestines markedly hemorrhagic; no other lesions except two small hemorrhages in the visceral pericardium, a number of small subendothelial hemorrhages on the ventricular side of the septum, and a large hemorrhage in a leaflet of the

aortal valve. Cultures from the blood on blood agar plates gave moderate number of small, gray, non-hemolysing colonies of diplococci, while the cultures in broth gave single cocci, diplococci, and chains of from five to ten members.

Rabbit 650.—Injected intravenously on April 14 with twenty-four-hour growth, from 35 c.c. of ascites dextrose broth, the organism resembling a streptococcus.

April 15, found dead: One small subcutaneous hemorrhage (3 by 8 mm.) around a blood vessel; pericardiac sac containing a moderate amount of turbid, slightly blood-tinged fluid; joint fluids distinctly turbid; one hemorrhage in the thymus gland; no other lesions. Cultures from the blood and joints gave a large number of gray, non-adherent, non-hemolysing colonies. Those from the pericardiac fluid gave a moderate number of colonies of cocci only.

Two other rabbits and one guinea-pig injected soon after the organism was isolated, showed skin lesions, but after cultivation for three weeks the strain lost this affinity for the skin as well as its virulence. After passing it through animals, the place of localization changed at the same time as its virulence increased and as the streptococcus forms displaced the bacilli.

Rabbit 638.—Injected intravenously on March 25 with the growth of the streptococcus from Dog 75 from 15 c.c. of ascites dextrose broth.

April 20, found dead: No gross lesions anywhere except those of the gall-bladder and liver. In the wall of the gall-bladder were twelve small, circumscribed, whitish areas. Smears from the turbid, slightly bile-tinged fluid in the gall-bladder gave large numbers of streptococci. The liver was soft, grayish-yellow in color, with areas of necrosis, smears from which showed a moderate number of gram-positive diplococci with leukocytes.

The streptococcus isolated from the gall-bladder in Rabbit 638 was injected intravenously into two rabbits, two guinea-pigs, and one dog. All the animals except one guinea-pig developed cholecystitis. Two showed hemorrhages in the mucous membrane of stomach or duodenum, two arthritis, and one myositis in addition. In one rabbit, one guinea-pig, and the dog, the organism acquired hemolytic power.

CASE 962.—Girl, 7 years of age, in the service of Dr. Rothstein and Dr. Walker at the Children's Memorial Hospital. There was no history of sore throat; measles one year ago; frequent convulsive—epileptic—seizures during past six months; appetite poor; constipated; slight blepharitis and conjunctivitis; throat and tonsils hyperemic; pyorrhea. The cultures from the throat containing diphtheria bacilli (?), 1,000 units antitoxin was given; no membrane. Several hyperemic papules appeared on arms and body. Throat became redder, both tonsils large, maxillary glands enlarged and tender, and three pea-sized, subcutaneous, bluish-red, painful and tender nodules appeared on the left forearm, one on right forearm, and several on the front of both legs; short, blowing, systolic murmur heard at apex and accentuation of pulmonary second. The tonsils were removed, followed by improvement of the lesion in the skin.

On April 22, cultures were made from blood, tonsil, and node excised twenty-two hours previously and kept on ice. The node was subsiding when excised. It yielded one small colony of a short diplobacillus-like organism in pure form; the overlying skin, two colonies of staphylococcus. The blood gave colonies of a non-hemolysing, short diplobacillus, resembling the one from the node. The tonsil yielded streptococci and clubbed, short diplobacilli.

A rabbit and a guinea-pig were injected intravenously with the mixed culture from the tonsil and a rabbit was injected with the pure culture from the blood. All developed circumscribed hemorrhages and edema of the subcutaneous tissues, the rabbits also slight arthritis and endocarditis. The guinea-pig, injected subcutaneously, showed marked edema, infiltration, and necrosis of the skin at the site of injection, also a mild arthritis, symmetrical hemorrhages of the tendinous attachment of muscles about both elbows, and subcutaneous hemorrhages over anterior aspect of both legs (Fig. 11).

CASE 32.—Boy, 9 years of age, in the service of Dr. Helmholtz at the Children's Memorial Hospital. He had painful, red, shining, circumscribed swellings on the front of the legs which came on suddenly with general malaise, fever, and sore throat, following severe wetting in a rain-storm eight days previously. The nodes on the legs appeared three days after the sore throat. The patient had had sore throat during the winter months. Tonsils were swollen.

Cultures from an excised node yielded three colonies of a diphtheroid bacillus. Tonsils were removed, and the emulsion made injected into animals.

A rabbit and one of two guinea-pigs injected with emulsion of the tonsils, showed hemorrhages in the skin. The rabbit showed, in addition, a few hemorrhages in the mucous membrane of the stomach and in the fascia of the muscles of the thorax. Diphtheroid bacilli and diplococci were isolated from the lesions.

CASE 51.—Woman, single, 21, service of Dr. Ormsby at the Presbyterian Hospital. Patient had had several attacks of rheumatism and an attack of diphtheria one month previously, when she was given antitoxin. Present illness began with malaise, fever, and an intense, persistent, frontal headache, which continued for two weeks, when there appeared painful, tender, red, indurated areas under the skin of the forearm and the front of legs and thighs, associated with a dull ache and distinct swelling in both elbow joints. The nodes appeared in successive crops. The fever ranged from 100 F. in the morning to 102 F. in the afternoon for nearly two weeks. As the nodes subsided, the temperature became normal. Tonsils were enlarged, pitted, and hyperemic; teeth decayed; the nodules in the skin well-defined, usually circular, bluish-red, from 0.5 to 6 cm. in diameter; leukocytes 13,000; urine showing moderate amount of albumin and hyalin casts, and an unusual number of leukocytes. Uneventful recovery.

The description of the microscopic appearances of a node in this case will serve to illustrate the appearances observed in others: Sections of the node showed no changes in the epidermis; the corium and subcutaneous tissue were the seat of extravasated blood, serous fluid, and a moderate leukocytic infiltration. In the center of the hemorrhagic area was a small artery plugged with leukocytes. Surrounding the vessel, and throughout the hemorrhagic area, were collections of blood pigment, which were easily seen with the low power and which were good places to search for bacteria, because it is here that they are most numerous (Fig. 3). One area in the subcutaneous tissue, just beneath the sweat glands, which showed no changes, presented dense leukocytic and round cell infiltration. Here only a few bacilli could be found, mostly within leukocytes. In all of the sections, areas of dense leukocytic infiltration were found usually situated near, or surrounding, a fair-sized artery. Thrombosis of the arteries in the center of or near areas of dense leukocytic infiltration was found in four of the cases; here bacilli were found in leukocytes within the lumen and in one within the vessel wall. The lymph channels were often dilated and filled with leukocytes (Fig. 5). The sequence of events appeared to be dilatation

and thrombosis of blood vessels and lymph channels, hemorrhage, polynuclear leukocytic infiltration, followed by invasion of plasma cells and endothelial leukocytes, the latter commonly mitotic. In no instance was there any evidence of invasion of the infection from epidermis or sweat glands. In only one case was there leukocytic infiltration around sweat glands, which were at the periphery of a large area in the subcutaneous tissue. The morphology of the organism found in the tissues corresponded quite accurately to that of those isolated in the cultures (Figs. 4, 5, 6, 17).

Owing partially at least to the fact that in the animals the tissue was removed sooner after the appearance of the lesions than in the patients, the hemorrhage was the marked feature and leukocytic infiltration relatively slight in the sections from the animals.

Here the hemorrhages and infiltration were nearly always in the deep layer of the corium, or loose subcutaneous tissue (Fig. 13). The hemorrhages usually surrounded a relatively large artery. In some of the sections the artery was the seat of thrombosis, or accumulation of leukocytes along the intima in the area of hemorrhages (Fig. 16). Some of the leukocytes contained organisms, and, in one instance, typical organisms were found directly in the wall of an artery which showed mural implantation of leukocytes (Fig. 17) adjacent to an area of hemorrhage in which exactly similar organisms were found in small numbers. Bacteria were never present in enormous numbers in the experimental lesions in the animals which were injected with the strains as isolated, and the amount of edema and leukocytic infiltration were correspondingly less than in the human lesions. However, after a number of animal passages, the lesions produced often showed the presence of many diplococci and chains (Fig. 14).

SUMMARY OF THE RESULTS AND GENERAL DISCUSSION

A diphtheroid, gram-staining, polymorphic, non-motile, non-spore-forming bacillus producing small, round colonies in dextrose agar, and small, gray, or yellowish, non-hemolysing colonies on blood agar, and having a wide range of fermentative power, was isolated from erythematous nodes removed in each of eight cases (Fig. 6). The number of colonies obtained ranged from 1 to 18,000. The nodes which were excised soon after their appearance, contained the largest number of bacteria, whereas those which had existed for from four to six days and where the symptoms were subsiding, contained fewer bacteria. A large proportion of the organisms were probably killed when the tissue was held in the Bunsen flame in sterilizing the surface, or perished

otherwise in the cultures, because the number of bacteria found in sections indicate a larger number present than is indicated by the number of colonies obtained. Thus in two sections which were examined of the node from Case 929, fully twice as many organisms were found as were indicated by the number of colonies which developed. One, two, and five colonies of *staphylococcus albus* were found in the node in three cases, and two colonies of *B. welchii* in one. In the rest, the organism appeared in pure form. The overlying skin, from which separate cultures were made in four cases, showed only three colonies of the diplobacillus and usually contained a few colonies of *staphylococcus albus*. The latter may be considered as of no etiologic importance, because it had no virulence and failed to produce skin lesions. The same organism as that obtained from the nodes was isolated at the same time in pure culture from the blood in two cases, and in conjunction with *B. welchii* in one case. The infection atrium would appear to have been the tonsils so far as the clinical history in two cases indicates; in three others the tonsils contained an organism which produced hemorrhages in the skin in animals; in another case the organism was found in a superficial ulcer in the throat, and in still another in an abscess of a tooth. In three cases the infection atrium was not apparent nor could it be determined. Constipation was present in these three cases. Throat infection was not severe at the time nodes developed, which usually was three to fourteen days after the throat symptoms were at their height. In four of the cases there was a definite but mild arthritis; in three, myositis or fibrositis; in two endocarditis; in four lymphadenitis; in two pericarditis; and in three, mild nephritis. All of the typical cases ended in recovery. The patient in whom the skin lesions were not typical and who suffered from a general invasion with the organism, died. Three cases occurred in children under 10 years of age, two girls and one boy. The other cases occurred in young women from 15 to 21 years of age. In two of the patients cultures from the throat showed bacilli indistinguishable from diphtheria bacilli; diphtheria antitoxin was given 3, and 28, days previously to the appearance of the nodes. The bacilli isolated from the nodes in these two cases were more like diphtheria bacilli than in the other cases and this circumstance suggests the possibility that the diphtheria bacillus may become so modified as to acquire affinity for the subcutaneous tissue.

In Table 1 is given a summary of the results in the animals which were injected intravenously with Strain 906. The figures in the column marked "Dose" indicate the growth from that many cubic centimeters of broth. The figure to the right and above the number of the strain injected, indicates the number of animal passages. It is seen that the organism soon after isolation has a marked affinity for the skin, which it loses on longer artificial cultivation as well as on animal passage. This fact was observed in the other cases as well and

TABLE 1
LESIONS PRODUCED BY INTRAVENOUS INJECTION OF STRAIN ISOLATED FROM CASE 906

Animal No.	Date Injected	Bacteria	Dose	Autopsy	Lesions in			
					Appendix	Stomach and Duodenum		G. Blac
						Hemor-rhage	Ulcer	
Rabbit 619	March 6, 7, 14	906 from node	30 c.c. 5 c.c. 5 c.c.	March 14	0	0	0	0
Rabbit 620	March 7.....	906 from node	5 c.c.	March 10	0	0	0	0
Rabbit 623	March 11.....	906 from node	7 c.c.	March 20	0	0	0	0
Dog 72....	March 11-14..	906 from node	60 c.c. 15 c.c.	March 17	0	0	0	0
Rabbit 624	March 12.....	906 ² from node	7 c.c.	March 19	0	0	0	0
Rabbit 627	March 14.....	906 from node	45 c.c.	March 20	0	0	0	0
Rabbit 629	March 16.....	906 ² from blood of Rabbit 619	7 c.c.	March 18	0	0	0	0
Rabbit 633	March 22.....	906 ³ from blood of Rabbit 629	6 c.c.	March 24	0	0	0	0
Rabbit 634	March 22.....	906 ² from blood of Rabbit 627	6 c.c.	March 24	0	0	0	0
Rabbit 639	March 26.....	906 ³ from blood of Rabbit 634	10 c.c.	March 28	0	0	0	0
Rabbit 640	March 26.....	906 ⁴ from blood of Rabbit 633	10 c.c.	March 27	0	0	0	0
Rabbit 642	March 28.....	906 ⁵ from blood of Rabbit 640	4 c.c.	March 31	0	0	0	0
Rabbit 645	April 6.....	906 from node..	30 c.c.	April 8	0	0	0	0
Rabbit 636	March 22.....	906 from node..	5 c.c.	March 28	0	0	0	0

found to hold with respect to the guinea-pig, rabbit, and dog. In Table 2 is given a summary of the results obtained with six strains injected intravenously when isolated and later. "When isolated" means soon after isolation, usually in the second or third culture. The tendency of the organism to localize in the subcutaneous tissue was seen in 18 of 20 animals injected with organisms soon after isolation, but in only 2 of 9 animals after cultivation for a longer time. The same strains after one to five animal passages, produced lesions in the skin in only 6 of 14 animals. These six animals were injected with strains

after only one or two animal passages, and the lesions were smaller and less numerous than those following injection of the strain when isolated. The shifting of the localization is well shown also in Table 1 in the case of Strain 906. This is not any accidental occurrence, because it was observed with all the strains and in different species. That it is due to acquired properties is illustrated in the results after injection of Strain 929,³ which was isolated from the bile in a rabbit with cholecystitis following injection of strain 929² and which pro-

TABLE 1—Continued
 LESIONS PRODUCED BY INTRAVENOUS INJECTION OF STRAIN ISOLATED FROM CASE 906

Lesions in									Remarks
Pan- creas	Joints	Endo- cardium	Myo- cardium	Muscle or Fascia	Kid- ney	Intes- tines	Lung	Skin	
0	0	0	0	0	0	0	0	+++	Regional lymph glands enlarged
0	0	0	0	0	+	0	0	++	Regional lymph glands enlarged
0	0	0	0	0	0	0	0	++	Regional lymph glands enlarged
0	0	0	0	0	0	0	0	++	Subcutaneous lymph glands enlarged
0	0	0	0	+	0	0	..	+	
0	0	0	0	0	0	0	0	+	
0	0	+	+	+	0	0	..	+	Lymph gland enlarged; hemorrhages in thymus and thyroid glands
0	+	+	0	+	0	0	0	+	Hemorrhages in liver
0	+	0	0	+	0	0	0	+	Hemorrhages in thyroid gland
0	++	0	0	+	0	0	0	0	
0	+++	0	0	0	0	0	..	0	Hemolytic streptococcus from blood and joints
0	+++	0	0	0	0	0	0	0	Pericarditis
0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	

duced cholecystitis in both of two rabbits, one dog, and in one of two guinea-pigs.

The four instances of arthritis and endocarditis following injection of strains when isolated were mild, while the arthritis which developed after animal passage was more marked. The renal lesions consisted of infarcts, chiefly in the medullary portion (Fig. 12), and subcapsular hemorrhages. The hemorrhages observed in the stomach or duodenum followed injection of the strain isolated from the acute ulcers in the fatal case (904), or after animal passage in the other strains.

TABLE 2
 LESIONS PRODUCED BY INTRAVENOUS INJECTION OF ORGANISM FROM ERYTHEMA NODOSUM WHEN ISOLATED AND AFTER
 ANIMAL PASSAGES AND ARTIFICIAL CULTIVATION

Condition of the Organisms	Number of Animals	Lesions in													
		Appendix	Stomach and Duodenum		Gall Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles of Fascia	Kidney	Intestines	Lung	Skin	Pericardium
			Hemorrhage	Ulcer											
When isolated	20	0	2	0	0	0	4	4	0	7	2	1	1	18	2
After one to five animal passages	14	0	3	0	7	0	7	2	2	7	1	1	6	6	1
After cultivation on artificial media for some time	9	0	2	0	1	0	1	1	0	0	0	0	0	2	0

The lesions in the subcutaneous tissue usually surrounded fair-sized blood vessels and were often symmetrically placed, especially in the extremities (Figs. 8, 9, 10, 11). They consisted first of circumscribed, rather large hemorrhages, which usually subsided without marked infiltration and edema, but in some instances there were redness of the skin and marked infiltration and edema as the hemorrhages faded. In some instances there were numerous small cutaneous lesions instead of the larger subcutaneous hemorrhage, resembling the hemorrhages in cases of "erythema multiforme." This result supports the view, held by some, that erythema multiforme is due to the same or similar cause as erythema nodosum. Subcutaneous injection in guinea-pigs of the strains as isolated, was followed by marked infiltration and necrosis of the skin at the point of injection. Intraperitoneal injection produced only slight lesions, the organisms being taken up promptly by leukocytes, but after animal passage, they produced serofibrinous peritonitis, the exudate showing little phagocytosis. Enlargement of the regional lymph glands occurred quite constantly in the animals in which lesions of the skin developed, and it made no difference whether the injection was made intravenously or subcutaneously. Cultures from the large hyperemic glands yielded the organism injected, as did the subcutaneous lesions (Figs. 14 and 15). The characteristic localization occurred after intravenous injection no matter whether the strain was isolated from the blood, the node, or the supposed infection atrium.

Since the organism resembles morphologically diphtheroid bacilli from other sources, a number of strains from Hodgkin's disease were injected as controls. None produced subcutaneous hemorrhages when injected intravenously, or infiltration when injected subcutaneously. Hemorrhages under the skin occur only rarely on intravenous injection of streptococci. An organism isolated from the thyroid gland in goiter resembles somewhat the one from erythema nodosum and hemorrhages under the skin have been observed in two rabbits following intravenous injection with the thyroid strain.

In three cases it was quite impossible to decide whether the organism isolated should be regarded as a streptococcus with marked involution forms, or as a diphtheroid bacillus with streptococcal forms. In the other cases the organisms when isolated seemed to belong to the diphtheroid group. In no instance was there a mixed infection with a bacillus and a streptococcus. On cultivation in some of the media and

on injection into animals, streptococcal forms were produced freely. The results obtained from a closer study of the character of two of the strains serve to illustrate the polymorphic character of the organism.

STRAIN 906.—Isolated from a cutaneous node and the blood. Subcultures on agar plates from four colonies gave small, moist, brownish-gray, non-adherent, non-hemolysing colonies with a distinct yellowish-green color on transmitted light. Smears showed many more short bacillary forms than in the original cultures, but there were all gradations between cocci and bacilli in the colonies examined. Subcultures on blood agar slants from two of the colonies were inoculated into ascites dextrose broth in tall tubes containing sterile kidney tissue at the bottom, and into dextrose broth. In both these developed diffuse, somewhat granular turbidity in twenty-four hours, smears showing organisms often in clumps similar to those in the dextrose agar except that bacillary forms were longer and narrower, and that distinct chains of cocci could be made out. Blood agar plates from these showed again the colonies first described, but smears from some of them showed bacilli only; others bacilli, elongated diplococci, and coccus forms; while still others showed coccus forms not only appearing in clumps, indistinguishable morphologically from staphylococci, but also in short chains of from five to ten members. Subcultures of the latter yielded cocci only, some of which were in chains, while those containing bacilli yielded bacillary forms only in ascites dextrose broth.

STRAIN 929.—Isolated in pure culture from a cutaneous node. Spherical bodies were present usually at one end of the bacillary form, but were found also in the center; there was also a rather large number of free coccus forms. The coccus forms retained Gram's stain more tenaciously than the rods and at times made the bacilli appear to be club-shaped and spore-bearing. Smears from the colonies near the top of the dextrose agar cultures showed the bacillary forms to be longer and narrower than those from the bottom of the tubes. All gradations in form between straight and clubbed bacilli, some of which retained Gram's stain while others did not, and gram-positive, elongated diplococci, and perfectly round coccus forms, were found in each of the ten colonies examined. Anaerobic cultures on Loeffler's serum which showed bacilli the day before and were incubated under aerobic conditions over night, presented most marked involution forms—numerous small cocci, and larger oval or round bodies both free, and in the middle, and at the end, of bacilli. The aerobic cultures on Loeffler's serum and blood agar showed a predominance of diplococcal forms often in short chains instead of bacilli. From one of the original colonies in the ascites dextrose agar there were inoculated ascites dextrose broth, with and without sterile tissue, and ascites dextrose agar in stab. The former yielded short, chain-forming cocci, while the broth containing the tissue yielded the bacillary forms only and the stab in ascites-dextrose-agar both varieties. Blood agar plates from ascites dextrose broth without tissue showed small, grayish, non-hemolysing, non-adherent colonies of small diplococci forming short chains, while the culture containing the tissue showed somewhat larger, brownish-yellow, more elevated, more opaque, non-hemolysing colonies containing bacillary forms only, which produce distinct foul odor.

Cultures were made from the blood in ten animals and from the subcutaneous lesions and enlarged adjacent lymph glands in seven animals, after intravenous injection with the organism in bacillary form. In six the blood yielded a pure culture of non-hemolysing coccoid forms in chains, in two bacillary and

streptococcal forms, and in two bacillary forms only; while the cultures from the subcutaneous node and lymph gland showed bacillary forms only in four, and both bacillary and streptococcal form in the other three. The strains isolated as bacilli after one animal passage assumed streptococcal form in the second animal passage (Figs. 6 and 7). That the streptococcal forms isolated from the animals really were changed bacillary forms and not accidental invaders from another source seems certain, because exactly similar streptococcal forms were isolated simultaneously in some instances from dog, rabbit, and guinea-pig.

Three of the strains of streptococcal form acquired hemolytic properties on animal passage. Strain 906 appearing as a non-hemolysing streptococcal organism after three animal passages, was injected into two rabbits, two guinea-pigs, and one dog. The blood or joint exudate from all yielded hemolysing streptococcal forms immediately after death from chloroform.

CONCLUSIONS

As a result of this study it appears that erythema nodosum is due to a diphtheroid bacillus, closely resembling in some stages the streptococcus group. This organism has an elective affinity for the subcutaneous tissues. The infection atrium and the place where the organism acquires the affinity for the skin appears commonly to be in the tonsils and pus pockets about the teeth. The reason for the pain in the cutaneous node is probably due to the fact that the hemorrhage, infiltration, and edema surround a relatively large blood vessel and hence the adjoining nerve trunk.

EXPLANATION OF PLATES 17 TO 22

PLATE 17

Fig. 1.—Cutaneous node from erythema nodosum in man (Case 51). Section showing infiltration of the subcutaneous tissue surrounding a thrombosed artery. Note the complete absence of involvement of cutis and only slight infiltration of the deeper layers of corium. $\times 17$.

Fig. 2.—Subcutaneous tissue from erythema nodosum in man (Case 906). Section showing marked leukocytic and round cell infiltration along the connective tissue strands between the layers of fat. $\times 53$.

PLATE 18

Fig. 3.—Subcutaneous tissue from erythema nodosum in man (Case 929). Section showing marked hemorrhage and beginning leukocytic infiltration and deposits of blood pigment.

Fig. 4.—Subcutaneous tissue from erythema nodosum in man (Case 929). Section showing red blood corpuscles, blood pigment, nuclei of disintegrated leukocytes and diplococci and diphtheroid bacilli.

Fig. 5.—Thrombosed artery in Fig. 1 showing a diphtheroid bacillus in the thrombus.

Fig. 6.—Smear from a single colony in ascites dextrose agar 72 hours after inoculation with the emulsion of the subcutaneous node in Case 929, showing diphtheroid bacilli.

Fig. 7.—Smear from blood of guinea pig injected with Strain 929, after one animal passage, showing typical diplococci in chains.

PLATE 19

Fig. 8.—Experimental erythema nodosum in rabbit. Skin showing an area of circumscribed subcutaneous hemorrhage and infiltration and an enlarged lymph gland, 72 hours after intravenous injection of the diphtheroid bacillus when isolated from the node in erythema nodosum in man (Case 906).

Fig. 9.—Experimental erythema nodosum in dog. Skin showing an area of circumscribed subcutaneous hemorrhage and infiltration, 72 hours after intravenous injection of the diphtheroid bacillus from the node in erythema nodosum in man (Case 906).

PLATE 20

Fig. 10.—Photograph showing circumscribed hemorrhages of the skin and symmetrical hemorrhages of the fascia of the interior aspect of the tibiae in rabbit, 48 hours after an intravenous injection of a diphtheroid bacillus from the subcutaneous node in erythema nodosum in man (Case 904).

Fig. 11.—Photograph showing symmetrical hemorrhages in the subcutaneous fascia of the elbows of guinea pig, 48 hours after intravenous injection of the diplobacillus from the blood in erythema nodosum in man (Case 962).

PLATE 21

Fig. 12.—Photograph of hemorrhagic infarct in the kidney and localized fibrositis and myositis over the thorax in a guinea pig, 72 hours after an intravenous injection of the strain from erythema nodosum after four animal passages.

Fig. 13.—Skin of rabbit. Section showing hemorrhage, and leukocytic and round cell infiltration of the subcutaneous tissue spaces, 72 hours after intravenous injection of the diphtheroid bacillus, see Fig. 8. Note the complete absence of involvement of the cutis and only slight infiltration in the corium.

PLATE 22

Fig. 14.—A diplococcus in the area of infiltration shown in Fig. 13.

Fig. 15.—Diplococci in a hemorrhagic lymph gland 48 hours after an intravenous injection of the diphtheroid bacillus after two animal passages.

Fig. 16.—Section of the artery from the area of subcutaneous hemorrhage shown in Fig. 9, showing mural aggregation of leukocytes.

Fig. 17.—Diplobacilli in the wall of the artery shown in Fig. 16.

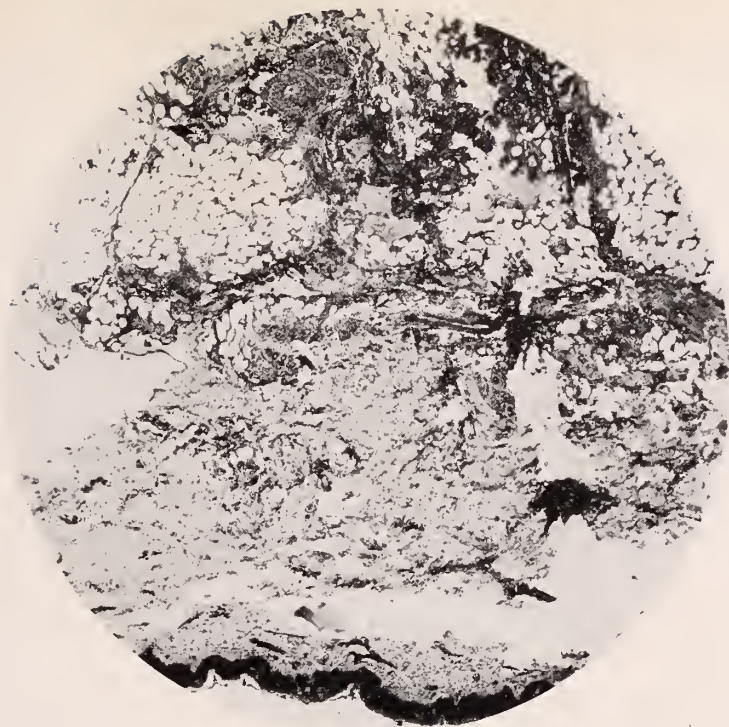


Fig. 1

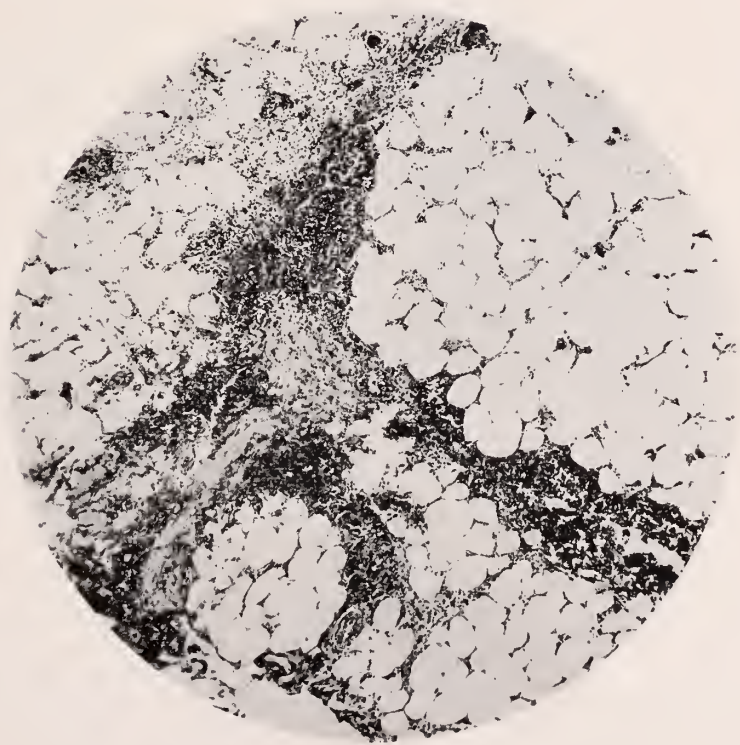


Fig. 2

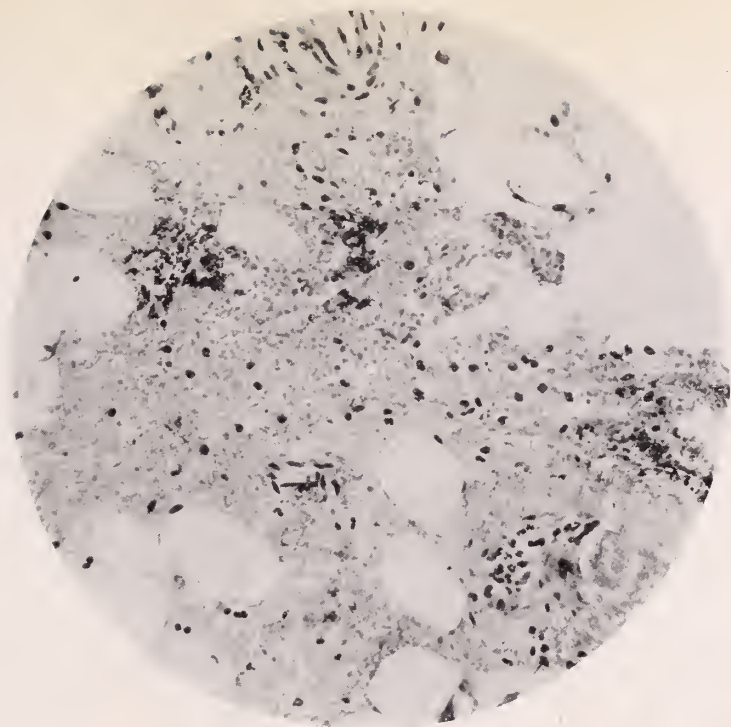


Fig. 3

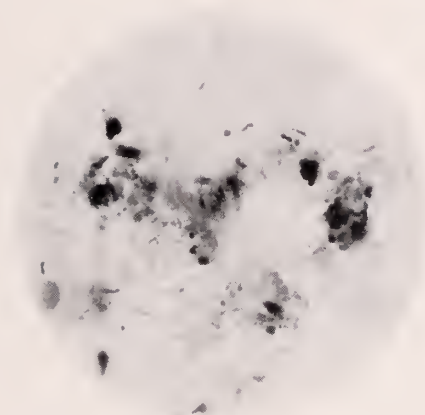


Fig. 4

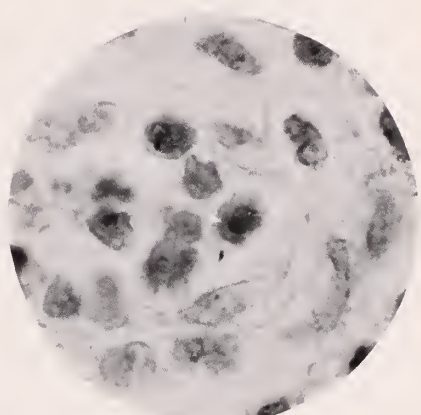


Fig. 5

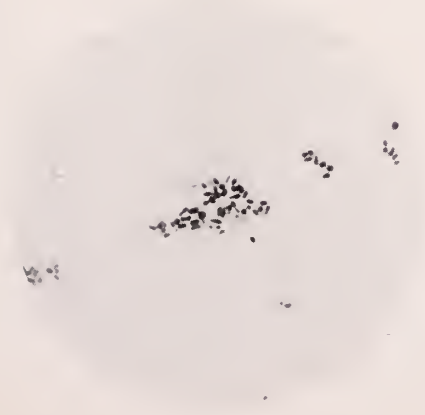


Fig. 6

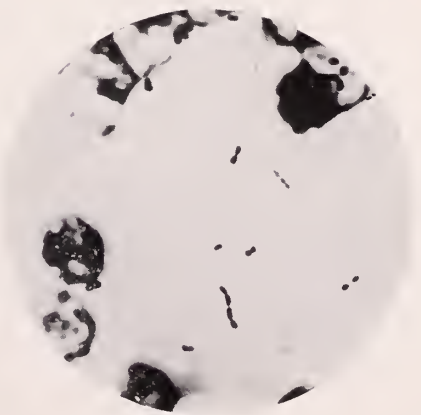


Fig. 7



Fig. 8

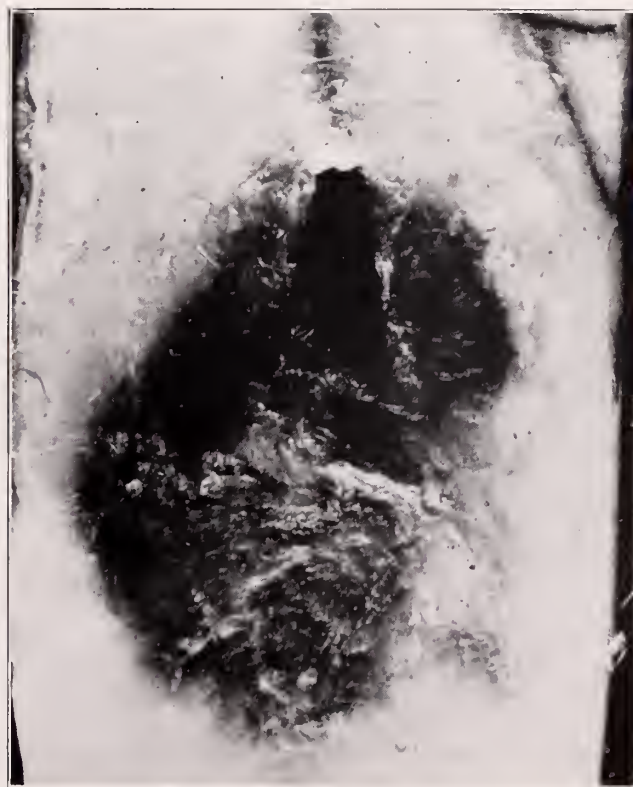


Fig. 9



Fig. 10

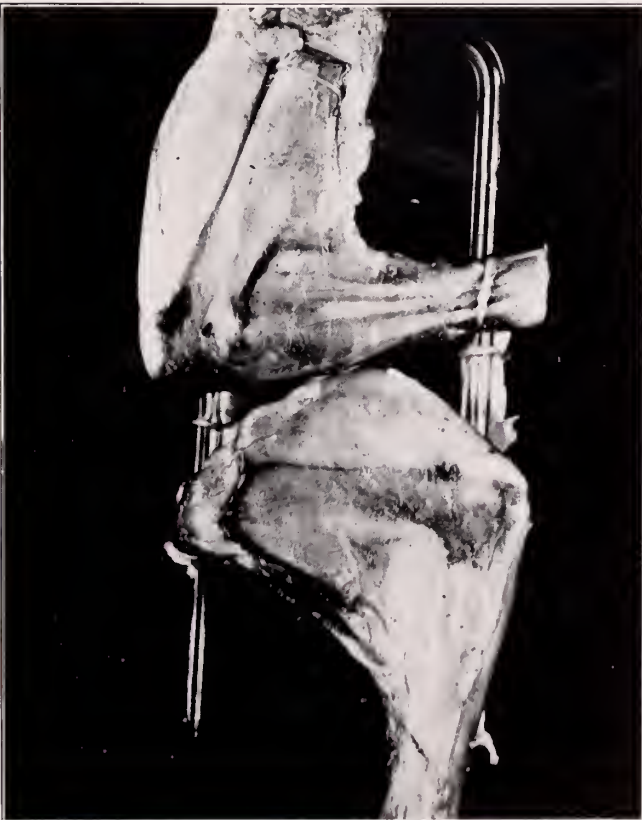


Fig. 11



Fig. 12

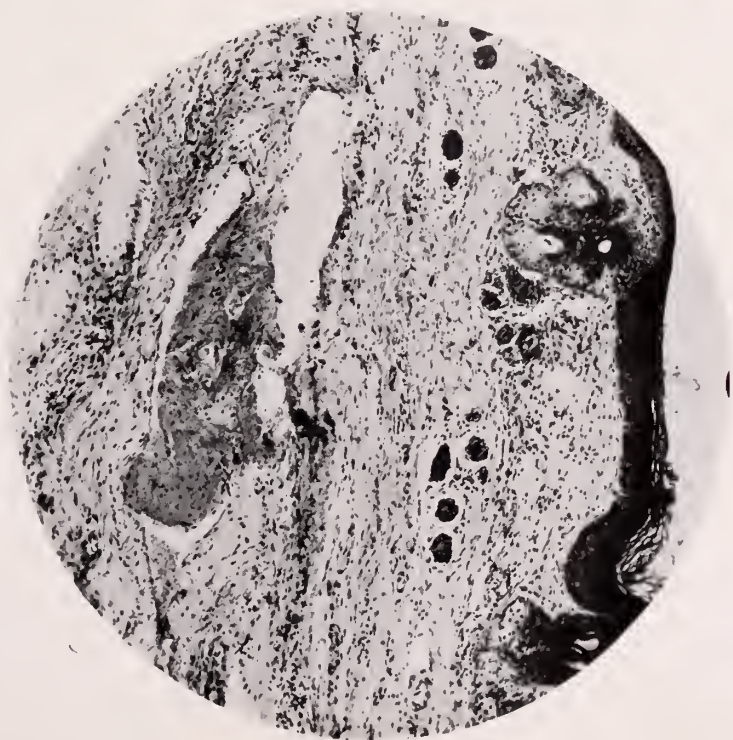


Fig. 13

PLATE 22

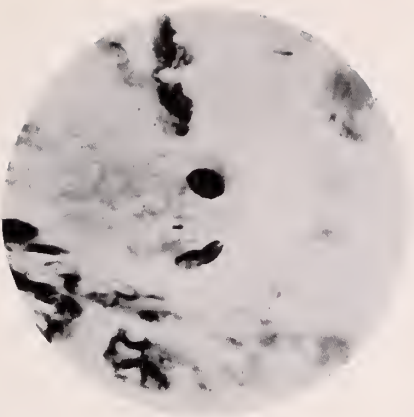


Fig. 14

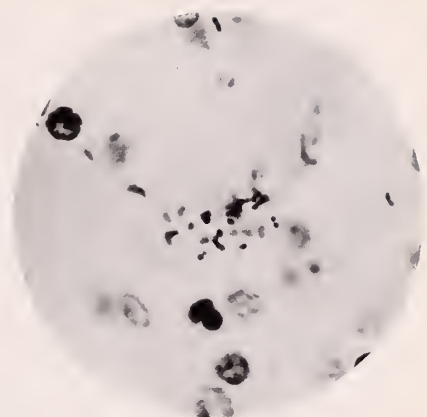


Fig. 15

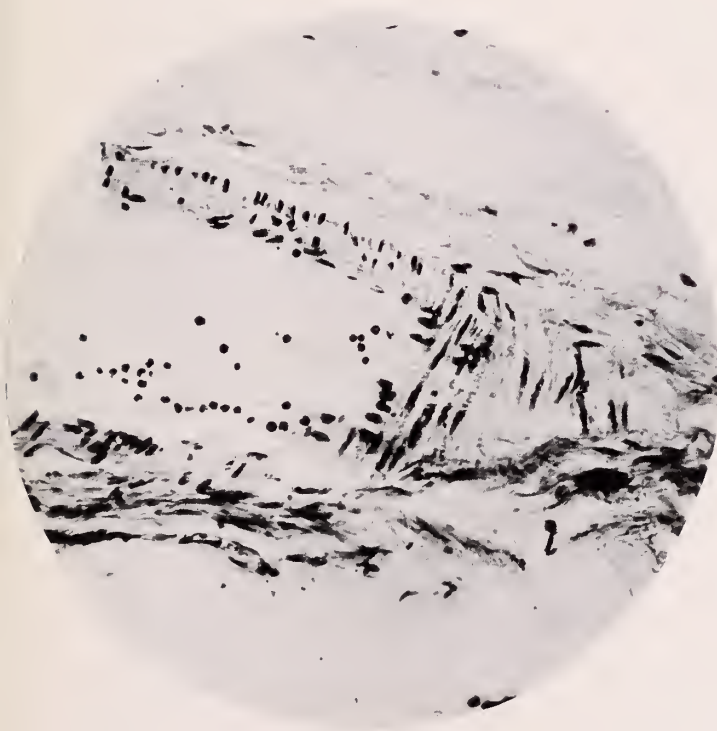


Fig. 16



Fig. 17

STARCH AGAR, A USEFUL CULTURE MEDIUM*

EDWARD B. VEDDER

Captain, Medical Corps, U. S. Army

(From the Laboratory of Pathology, Army Medical School, Washington, D. C.)

The medium about to be described was devised in the course of experiments to obtain a more suitable medium for the cultivation of the gonococcus. We needed to keep in stock a number of cultures of gonococci for the preparation of vaccine and antigen for the gonococcus fixation test. We were using salt-free veal agar prepared according to the method used by the New York Board of Health. This medium possesses many advantages, but the transfers must be made every two or three days in order to keep the cultures alive, and we desired a medium on which gonococci could remain alive for a longer period in order to avoid the necessity for such frequent transfers.

The actual work in these experiments was performed chiefly by one of our laboratory assistants, Sergeant Frederick G. Abner, and it is only fair to state that the results here presented are due to the interest he has taken in this work.

The medium on which we have had the best success in cultivating the gonococcus consists of beef infusion agar to which 1 percent of starch has been added.

METHOD OF PREPARATION

Beef infusion is prepared according to the usual method employed for making broth. It is essential to use a beef infusion because commercial meat extracts do not give as good results. To this beef infusion sufficient agar is added to make 1.5-1.75 percent agar. No peptone or salt is added, since the gonococcus appears to grow better on media without peptone or salt. If much more than this amount of agar is added, the medium will be too dry.

This mixture is cooked, clarified, and filtered according to the method of preparing ordinary agar. It is then neutralized so that the final reaction will be from 0.2-0.5 percent acid to phenolphthalein. Ten grams of cornstarch to each liter are now added to this medium. It is well to grind up the cornstarch in a mortar with a little of the

* Received for publication February 13, 1915.

agar in order to avoid lumps. We have tested this medium using varying amounts of cornstarch and have found that about 1 percent is the optimum amount. After the addition of the cornstarch, the mixture is boiled for a few minutes and this concludes the preparation of the medium, which is then tubed and sterilized at not more than fifteen pounds pressure to avoid breaking down the starch. The medium so prepared will be slightly clouded owing to the presence of the cornstarch.

We have tested other kinds of starch in this culture medium in order to determine whether starch from any source was equally suitable. For this purpose we have used tapioca, potato starch, and wheat starch. The gonococci grew on all of these starch agars better than on the ordinary media employed for the cultivation of these organisms, but the growth appeared to be less luxuriant than when cornstarch was used. For the most part, cornstarch appeared to be more suitable for other organisms also. Kahlbaum's cornstarch for medicinal use was first tried, but ordinary commercial cornstarch appeared to give as good results and is of course much cheaper. The tapioca and the potato starch media, while not producing so luxuriant a growth, have not the slight clouding that appears unavoidable when cornstarch is used.

ADVANTAGES OF THIS MEDIUM

Starch agar medium has proved to be the best medium that we have tried for the cultivation of the gonococcus. The growth of this organism is so profuse that it would lead us to suspect that the organism is not really the gonococcus were it not for the fact that our work has been done with the original Torrey strains of this organism, which were kindly furnished us by the New York Board of Health. It also might be supposed that this profuse growth is obtained because the gonococci used are old strains that have been under cultivation for a long time, but we have also been successful in isolating gonococci from several fresh cases of gonorrhea on this medium, and the strains so isolated also grow freely. There is free growth after twenty-four hours' incubation and this increases up to three or four days, after which there is no apparent increase in the density of the growth. Cultures on this medium are suitable for the preparation of antigen, as the starch appears to have no bad effect and the dense growth is a distinct advantage.

Gonococci remain alive upon this medium for a long time. It has been our custom to remove the cultures from the incubator after several days and to keep them thereafter at room temperature. Experiments have been made to determine how long the gonococci will remain alive under these circumstances. In one of these experiments, out of seven Torrey strains that were kept for forty days, five of the seven were alive at the end of this time. We have found that they remain alive with great regularity for twenty days, and that we can safely make transfers of our stock cultures every two weeks, instead of every two or three days as is necessary when salt-free veal agar is used.

This medium may be melted and used in plates to plate out cultures of gonococci, or the pus from a fresh case of gonorrhea may be smeared on the surface of a plate and the gonococcus isolated from the colonies that develop.

Some other organisms that are usually cultivated with some difficulty grow readily on this medium. We have tried a number of strains of tubercle bacilli and one of the possible lepra bacilli isolated by Duval, and all of these organisms grew freely on this medium. Freshly isolated streptococci and pneumococci also grow more freely than on the other media.

The medium is suitable also for routine use. Practically all of our stock cultures grew either as well or more luxuriantly on this starch medium than on plain agar.

The great simplicity of this medium and its easy preparation are strong recommendations. It is even easier to prepare than salt-free veal agar because it is easier to obtain beef than veal at a certain time and no peptone is required.

A starch agar, prepared by a different method with potato starch, has been used by several workers¹ in the study of certain soil bacteria, and starch has also been used in various ways by general bacteriologists, but, so far as we are aware, no one has heretofore used a starch agar prepared as described here for the cultivation of the gonococcus. It has been known for some time that a considerable number of bacteria produce amylases, or ferments capable of breaking down starch, and that by cultivating certain bacteria upon starch agar plates the amylase can be demonstrated by the clearing of the medium immediately surrounding the colonies. This effect is seen very clearly on plates on

1. McBeth and Scales: U. S. Dept. of Agriculture, Bureau of Plant Industry, Bull. 266, 1913; Kellerman and McBeth: *Centralbl. f. Bakteriologie*, I, O., 1912, 34, p. 487.

this starch medium sown with cholera spirilli, but colonies of the gonococcus rarely present this appearance. In spite of this fact, it seems evident that the gonococcus is able to utilize the starch.

Why should starch serve better as a culture medium than the various sugars? It might be assumed, since starch must be broken down into sugars before it is available for absorption by an organism that, therefore, nothing could be expected of starch in a culture medium which would not be obtained equally well from the use of sugars. This appears not to be the case however. Gonococci grow better on this starch medium than upon any sugar medium that we have tried. Just why starch should produce this effect remains a subject for further study. It may be that when starch is acted upon by the amylases peculiar to the organism, or in other words is subject to the normal process of digestion, that the resultant products of decomposition are more suitable for the growth of the organism than is the case when certain sugars are furnished. But whatever the explanation, it appears that this starch agar is a simple culture medium specially suitable for the cultivation of a number of bacteria that have hitherto required rather complicated culture media.

COMPLEMENT-FIXATION IN WHOOPING COUGH *

WALTER WINHOLT

(From the Memorial Institute for Infectious Diseases, Chicago)

INTRODUCTION

Since Bordet and Gengou¹ described the bacillus of pertussis in 1906 as the specific cause of whooping-cough, a number of investigators have studied the organism with respect to complement-fixation. Bordet and Gengou claim that this bacillus is the cause of whooping-cough, because it is found in overwhelming numbers during the early course of the disease, and because it gives complement-fixation with the serum of pertussis patients, this reaction not occurring with any other organism associated with the disease. However, according to some writers, there are several points which are not clearly demonstrated. In the first place, it is said that we lack sufficient evidence that this organism is present in every case of pertussis. The bacillus usually can be isolated in the early stages of pertussis, but after the second week it is not present in the sputum in sufficiently large numbers to be readily isolated. The influenza bacillus, on the other hand, can be isolated as early in whooping-cough as the Bordet-Gengou bacillus, and is present for a longer time. Secondly, complement-fixation with the serum of patients is said not to be as clear cut as it seems to be with the serum of animals which have been injected with the bacillus of pertussis. Finally, the results obtained with specific vaccines in treatment and prevention have not been altogether satisfactory. Thus Hartshorn and Moeller,² in reviewing the literature on the use of pertussis vaccines, conclude that its prophylactic value is still undetermined.

THE BACILLUS PERTUSSIS

The bacillus pertussis has been carefully described by the discoverers and numerous investigators have confirmed their observations. It is a small, short, ovoid, non-motile bacillus, which does not form spores, and which does not have a capsule. It is said to be slightly larger than

* Received for publication March 12, 1915.

Aided by a grant from The Fenger Memorial Fund.

1. Ann. de l'Inst. Pasteur, 1906, 20, p. 731.

2. Arch. Pediat., 1914, 31, p. 580.

the influenza bacillus. It stains well with methylene blue, dilute carbolfuchsin, and carbol-gentian-violet, the poles being frequently more deeply stained than the center. Usually the bacilli are separate and single, but they may appear in pairs and in chains.

Bordet and Gengou use a glycerin potato blood agar medium, but the organism grows well on ascitic agar, potato blood agar, and in ordinary blood agar. There seems to be little choice in the blood—human, rabbit, goat, or pigeon blood being used by different workers with equally good results.

On the potato glycerin blood agar, the growth is barely visible in twenty-four hours. In forty-eight hours the colonies have a gray, glistening appearance. The growth increases in thickness rather than in size. At times the growth assumes a pale blue color. The organism does not blacken the medium. On blood agar the growth is thin, and somewhat slow. The organism does not grow on plain agar, in litmus, milk, or broth. A few drops of blood in the broth make a good medium. The broth cultures containing a few drops of blood grow best when the tubes are kept in a slanting position, because the organism is such a strict aerobe. It is killed when heated to 56 C. for thirty minutes.

Morphologically, the influenza bacillus resembles the bacillus pertussis, the former being regarded as slightly smaller. Like the bacillus pertussis it is gram-negative, does not form spores, and is aerobic. It frequently stains more deeply at the ends than in the center. It requires hemoglobin for growth. On potato glycerin blood agar, the growth, after the first generation, is not as vigorous as the growth of the bacillus pertussis. When the organism is suspended in water, it gives an emulsion with a tendency to spontaneous agglutination, so that in drying on the slide the bacilli are frequently clumped in small masses. In preparations of whooping-cough bacillus, the organisms remain separated. The influenza bacillus frequently darkens the color of blood media, and this Bordet and Gengou³ regard as distinctive. On ascitic agar the whooping-cough bacillus grows slowly, as a white streak, while the influenza bacillus grows only very slightly, and not as well as the bacillus pertussis.

The influenza bacillus can be differentiated from the bacillus pertussis also by agglutination and complement-fixation. The serum of rabbits injected with the Bordet-Gengou bacillus, agglutinates the

organism in dilutions of 1-800 or higher, but has no effect on the influenza bacillus. The serum of rabbits injected with the influenza bacillus, agglutinates this organism in dilutions of 1-500 or so, and has no effect on the pertussis organism.

Moreover, positive complement-fixation is obtained by using the bacillus pertussis as antigen with the serum of rabbits injected with the organism, while negative results are obtained when the influenza bacillus is used as antigen with such serum. When the serum of rabbits injected with influenza bacilli is used, positive complement-fixation is obtained with the influenza bacillus as antigen; negative results with the bacillus of pertussis.

COMPLEMENT-FIXATION IN WHOOPING-COUGH

The work on complement-fixation with the bacillus of pertussis has brought varied results, perhaps in part due to the different methods used to prepare the antigen.

Bordet and Gengou used suspensions in salt solution of growths on solid media. Wollstein⁴ used three forms of antigen: suspensions of the bacilli in salt solution; extracts of bacilli made by suspending the growths of three blood agar slants in 5 c.c. of salt solution and shaking for twenty-four hours in thermostat; and extracts of tissue obtained from patients dying from pertussis. Friedlander and Wagner⁵ used live bacteria and fresh serum, and considered this innovation of great importance. The different hemolytic systems employed may also have had some bearing on the failure to obtain identical results.

Bordet and Gengou obtained complement-fixation in all of their cases of pertussis. They used 0.1 c.c. to 0.3 c.c. of the human serum heated to 56 C., and 0.05 c.c. to 0.01 c.c. of fresh guinea-pig serum as complement. The amboceptor was the serum of rabbits previously injected with sheep corpuscles. The antigen was an emulsion of the bacillus of pertussis in salt solution, the growth being twenty-four hours old.

Arnheim⁶ obtained complement-fixation in six of twelve cases of pertussis. Wollstein examined the serum from nine patients with pertussis and in no instance did she obtain complement-fixation, using the three forms of antigen just mentioned in all cases. The quantities of complement and amboceptor were about the same as those used by Bordet and Gengou.

Gengou and Brunard⁷ describe three cases in which they determined the specific pertussis character of the infection by complement-fixation.

In 1911 Bordet and Gengou⁸ reported certain atypical cases diagnosed as pertussis by means of complement-fixation. A little later Bordet⁹ concluded that the power to fix complement is not found early, and does not become marked until near the end of the disease.

4. Jour. Exper. Med., 1909, 11, p. 41.

5. Am. Jour. Dis. of Children, 1914, 8, p. 134.

6. Berl. klin. Wchnschr., 1908, 14, p. 1453.

7. Bull. Acad. de med. Belg., 1910, 24, p. 329.

8. Centralbl. f. Bakteriöl., I, O., 1911, 58, p. 573.

9. Ibid., 1912, 66, p. 275.

St. Bächer and Menschikoff¹⁰ report twenty-seven cases of pertussis in all stages in which attempts were made to obtain complement-fixation without success in a single case. Only after vaccines of pure cultures of the bacillus pertussis were given, did fixation occur. Their antigen was an emulsion of the bacillus of pertussis in salt solution, while 0.4 c.c. of a 1/10 dilution of guinea-pig serum served as complement, and 0.5 c.c. of a 1/150 dilution of serum of a rabbit previously injected with sheep corpuscles served as amboceptor.

Delcourt¹¹ obtained complement-fixation in six cases of pertussis. Poleff¹² gives a résumé of the results of five investigators. In five cases only was there complement-fixation and in thirty-one cases no fixation was obtained. He, himself, reports ten cases in which he did not obtain fixation.

Hess¹³ tested ten cases and concluded "that results would seem to show that this reaction is present for some months after cessation of all symptoms."

Recently Anna Wessels Williams¹⁴ came to the conclusion that the test with serum of human beings is not as clear cut as it seems to be with serum of animals which have been injected with the bacillus of pertussis.

Renaux,¹⁵ using the bacillus pertussis for antigen, examined seventy-three sera for complement-fixation, thirty-two cases of which were known cases of pertussis. Of the cases of pertussis, he obtained complement-fixation in twenty-three. Of the nine negative cases, the serum had been obtained early during the disease, and three of these gave positive complement-fixation when the attack had lasted about four weeks. No further examination was made on the remaining six cases. His results seem to show that fixation appears about three or four weeks after the appearance of the whoop.

In eighteen cases of pertussis, Friedlander and Wagner¹⁶ obtained complement-fixation in each case. They used fresh serum of pertussis patients and living bacteria. The Noguchi hemolytic system was used throughout their work on account of the small quantity of serum necessary. They claim that the diagnosis of pertussis can be made with certainty in the catarrhal stage by means of complement-fixation. In a more recent article, Friedlander¹⁷ reports further results. He obtained fixation in thirteen of fourteen cases in the catarrhal stage before the characteristic whoop had appeared. In one case there was fixation three weeks before the first whoop.

This review shows that varied results have been obtained with complement-fixation in pertussis. The reaction is regarded as most pronounced late in the disease by Bordet and Gengou. Several investigators have found the reaction present after the cessation of all symptoms. Then, again, negative results have been obtained by Wollstein, St. Bächer, and Menschikoff, and others. Positive fixation has been obtained before the appearance of the whoop by Friedlander and Wagner.

10. *Ibid.*, 61, p. 218.

11. *Presse. med. Belg.*, 1912, 64, p. 19.

12. *Centralbl. f. Bakteriöl.*, I, O., 1913, 69, p. 23.

13. *Jour. Am. Med. Assn.*, 1914, 63, p. 1007.

14. *Arch. Pediat.*, 1914, 31, p. 567.

15. *Centralbl. f. Bakteriöl.*, I, O., 1914, 75, p. 197.

16. *Am. Jour. Dis. of Children*, 1914, 8, p. 134.

17. *Lancet-Clinic*, 1915, 113, p. 8.

AGGLUTINATION IN WHOOPING-COUGH

It has been found that the serum of children with pertussis agglutinates the bacillus of pertussis in varying concentrations.

Wollstein found that the serum of animals injected with the bacillus of pertussis agglutinates the organism in dilutions as high as 1-800, while no agglutination of the pertussis organism results with the serum of animals immunized with the influenza bacillus. The serum of animals immunized with the influenza bacillus agglutinates this bacillus in dilutions of 1-400.

Bordet and Gengou found that agglutination took place on the addition of 0.002 c.c. of the serum of a horse immunized with the bacillus of pertussis to 1 c.c. of an emulsion of the pertussis organism. They further state that their horse serum agglutinated the strain that was used to immunize the animal less than it did another strain from a different case of pertussis.

Practically all agree that serum from patients suffering with pertussis show very inconstant agglutinative properties.

PERSONAL OBSERVATIONS

Due to the close resemblance between the bacillus pertussis and the influenza bacillus, I have made further experiments to study the relationship, if any exists, between these two organisms themselves on the one hand, and whooping-cough on the other, so far as determinable by means of complement-fixation and agglutination.

Bacteriological examinations were made on cases of whooping-cough in which the whoop was still present, and the bacillus pertussis was isolated from three cases early in the disease. These strains corresponded to the description given by Bordet and Gengou of the bacillus pertussis. They corresponded also to the cultures from the American Museum in all ways, except that they were not so easily agglutinated. No agglutination or complement-fixation was obtained with these strains and the serum of rabbits immunized with the influenza bacillus. It may be mentioned that normal rabbit serum frequently agglutinates the pertussis organisms in dilution of 1-20 or 1-30.

The culture of the bacillus pertussis obtained from the American Museum of Natural History, New York, answered in every way to the description given by Bordet and Gengou. After a number of subcultures had been made, this organism grew readily on blood agar.

In all, fifty-one cases have been studied, twenty-two cases with a history of pertussis, and twenty-nine cases without any symptoms or recent history of pertussis.

Except for a few minor changes the test for complement-fixation has been made as described by Bordet and Gengou. The patient's blood was allowed to coagulate, the serum separated in the centrifuge and heated to 56 C. for twenty minutes. In the tests 0.01 c.c. to 0.04 c.c. of the undiluted serum was used. The amboceptor consisted of antsheep rabbit serum, diluted 1-10. The antigens consisted of seventy-two-hour growths of the bacillus of pertussis on potato glycerin blood agar and growths of the influenza bacillus on blood agar, the growths being washed off into normal salt solution and the suspensions heated to 56 C. for thirty minutes.

TABLE 1
TITRATION OF COMPLEMENT AND AMBOCEPTOR

Complement 1-10	Amboceptor 1-100	Hemolysis
0.25 c.c.	0.3 c.c.	Complete
0.125 c.c.	0.3 c.c.	Complete
0.06 c.c.	0.3 c.c.	Complete
0.03 c.c.	0.3 c.c.	Partial
0.01 c.c.	0.3 c.c.	Slight
.....	0.6 c.c.	None
0.06 c.c.	0.15 c.c.	Complete
0.06 c.c.	0.08 c.c.	Complete
0.06 c.c.	0.04 c.c.	Partial
0.06 c.c.	0.01 c.c.	Slight
0.06 c.c.	None
Salt solution control	None

Each tube contains 0.35 percent sheep corpuscles. Total quantity in each tube = 1 c.c.

Early in the work, the sera of rabbits previously injected with gradually increasing doses of bacillus pertussis were used for positive controls. The growths on two slants of potato glycerin blood agar were injected intraperitoneally. In three days a second injection was made with growth on these slants. Such injections were made every three days five times. At the end of this time strong fixation occurred. At intervals of four weeks, a single intraperitoneal injection was made, a strongly positive serum being thus maintained. In a similar way, rabbits were given intraperitoneal injections of the bacillus influenzae in order to get a good positive serum.

Complement and amboceptor were titrated in the usual way, as shown in Table 1. The method of titration of antigen is shown in Table 2. Various amounts of patients' serum were used in the fixation tests.

TABLE 2
TITRATION OF ANTIGEN

Antigen	Antiserum	Hemolysis Antigen + Antiserum + Complement 1-10, 0.125 c.c. (Total Quantity 0.5 c.c.) Incubated at 37 C. for One Hour; then Amboceptor 0.15 c.c. and 5 Percent Sheep Corpuscles 0.3 c.c. Added to Each Tube. Total Quantity 1 c.c.
Suspension B. Pertussis 0.25 c.c.	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Pertussis 0.15 c.c.	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Pertussis 0.08 c.c.	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Pertussis 0.03 c.c.	Normal Rabbit Serum .04 c.c.	Complete
.....	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Pertussis 0.3 c.c.	Complete
Suspension B. Pertussis 0.3 c.c.	None (No amboceptor)
Suspension B. Pertussis 0.25 c.c.	Antipertussis Serum .04 c.c.	None
Suspension B. Pertussis 0.15 c.c.	Antipertussis Serum .04 c.c.	None
Suspension B. Pertussis 0.08 c.c.	Antipertussis Serum .04 c.c.	None
Suspension B. Pertussis 0.03 c.c.	Antipertussis Serum .04 c.c.	Slight
.....	Antipertussis Serum .04 c.c.	Complete
Suspension B. Influenzae 0.25 c.c.	Complete
Suspension B. Influenzae 0.15 c.c.	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Influenzae 0.08 c.c.	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Influenzae 0.03 c.c.	Normal Rabbit Serum .04 c.c.	Complete
.....	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Influenzae 0.3 c.c.	Normal Rabbit Serum .04 c.c.	Complete
Suspension B. Influenzae 0.3 c.c.	None (No amboceptor)
Suspension B. Influenzae 0.25 c.c.	Anti-influenza Serum .04 c.c.	None
Suspension B. Influenzae 0.15 c.c.	Anti-influenza Serum .04 c.c.	None
Suspension B. Influenzae 0.08 c.c.	Anti-influenza Serum .04 c.c.	None
Suspension B. Influenzae 0.03 c.c.	Anti-influenza Serum .04 c.c.	Slight
.....	Anti-influenza Serum .04 c.c.	Complete

TABLE 3
COMPLEMENT-FIXATION TESTS

Antiserum	Antigen	Hemolysis Antiserum + Antigen + Complement 1-10, 0.125 c.c. (Total Quantity 0.5 c.c.) Incubated at 37 C. One Hour; then Amboceptor 0.15 c.c. and 5 Percent Suspension of Sheep Corpuscles 0.3 c.c. Added to Each Tube, the Total Quantity Being Brought up to 1 c.c.
Antipertussis Rabbit Serum .04 c.c.	Suspension B. Pertussis 0.15 c.c.	None
Antipertussis Rabbit Serum .02 c.c.	Suspension B. Pertussis 0.15 c.c.	None
Antipertussis Rabbit Serum .01 c.c.	Suspension B. Pertussis 0.15 c.c.	None
Antipertussis Rabbit Serum .06 c.c.	Complete
Normal Human Serum .04 c.c.	Suspension B. Pertussis 0.15 c.c.	Complete
Normal Human Serum .02 c.c.	Suspension B. Pertussis 0.15 c.c.	Complete
Normal Human Serum .01 c.c.	Suspension B. Pertussis 0.15 c.c.	Slight
Normal Human Serum .06 c.c.	Complete
Pertussis Patient Serum .04 c.c.	Suspension B. Pertussis 0.15 c.c.	None
Pertussis Patient Serum .02 c.c.	Suspension B. Pertussis 0.15 c.c.	None
Pertussis Patient Serum .01 c.c.	Suspension B. Pertussis 0.15 c.c.	None
Pertussis Patient Serum .06 c.c.	Complete
Anti-influenza Rabbit Serum .04 c.c.	Suspension B. Influenzae 0.25 c.c.	None
Anti-influenza Rabbit Serum .02 c.c.	Suspension B. Influenzae 0.25 c.c.	None
Anti-influenza Rabbit Serum .01 c.c.	Suspension B. Influenzae 0.25 c.c.	None
Anti-influenza Rabbit Serum .06 c.c.	Complete
Pertussis Patient Serum .04 c.c.	Suspension B. Influenzae 0.25 c.c.	Complete
Pertussis Patient Serum .02 c.c.	Suspension B. Influenzae 0.25 c.c.	Complete
Pertussis Patient Serum .01 c.c.	Suspension B. Influenzae 0.25 c.c.	Complete
Pertussis Patient Serum .06 c.c.	Complete

A full set of controls was made with every test. Tests for anti-complementary properties and for natural amboceptor were made always. By using the Bordet-Gengou bacillus and the influenza bacillus as antigens, complement-fixation tests were made in twenty-eight cases without any symptoms or history of pertussis. This series included normal persons, patients with measles, scarlet fever, chicken-pox, and bronchitis. In no case was there any fixation of complement with either the pertussis organism or the influenza bacillus, the hemolysis being always complete. This series included three persons who had had pertussis two years, three years, and five years previously, but no fixation was observed.

TABLE 4

COMPLEMENT-FIXATION AND AGGLUTINATION TESTS WITH *BACILLUS PERTUSSIS* AND *BACILLUS INFLUENZAE* IN WHOOP COUGH

Number	Age	Duration of Disease	Complement-Fixation		Agglutination	
			B. Pertussis	B. Influenzae	B. Pertussis	B. Influenzae
1	17 months	3 months	Complete	None	0	0
2	5 years	14 days	Complete	None	1-70	0
3	4 years	2 months	Complete	None	1-60	1-20
4	4 years	20 days	Partial	None	1-80	1-10
5	7 years	6 weeks	Complete	None	1-40	0
6	6 months	4 weeks	Partial	None	1-10	1-10
7	2 years	4 months	Partial	None	1-50	1-10
8	3 years	18 months	Partial	None	0	0
9	6 years	25 days	Complete	None	1-150	1-30
10	2 years	14 days	Partial	None	1-240	0
11	3 years	14 days	Slight	None	1-120	1-30
12	1 year	4 months	Slight	None	1-20	1-10
13	7 years	3 weeks	Slight	None	1-60	0
14	5 years	3 weeks	Slight	None	1-50	0
15	6 years	3 weeks	Complete	None	1-60	1-10
16	3 years	2 weeks	Partial	None	1-10	0
17	4 years	2 weeks	Slight	None	1-30	0
18	3 years	2 weeks	Slight	None	1-30	0
19	6 years	1 day	Slight	None	0	0
20	8 years	3 months	Complete	None	1-70	1-10
21	7 years	8 months	Slight	None	0	0
22	2 years	4 months	Complete	None	1-10	0

Twenty-two persons with active pertussis or history of a recent attack were tested for complement-fixation. Serum was obtained at various times during the attack and also from persons who had had pertussis eighteen months before. All the cases of pertussis examined at about two weeks after the onset of the whoop gave complement-fixation, fixation being not absolutely complete but very definite and distinct. At later periods the fixation became more marked. Six to eight weeks after the onset of the disease the complement-fixation was always complete. At three months or so after the onset of the

attack, the fixation of the complement was still complete. At eight months the reaction was present but not as marked as at three months. One case which gave a history of severe pertussis eighteen months previously showed a slight fixation. Persons who had had pertussis two, three, and five years previously showed no fixation. Serum was obtained from one case at the very onset of the attack, but there was neither fixation at this time, nor nine days after onset. At eighteen days after the beginning of the whoop, there was a slight but distinct fixation.

When the influenza bacillus was used as antigen, all the sera that gave positive results with the bacillus pertussis, gave uniformly negative results.

AGGLUTINATION

The serum of rabbits injected with the bacillus of pertussis agglutinated the organism in dilutions of 1-1,000, but had no effect on the influenza bacillus. The serum of rabbits injected with the influenza bacillus agglutinated this organism in dilutions of 1-500, but had no effect on the pertussis organism.

The serum of the patients with pertussis, or convalescing therefrom, showed variable agglutinating properties towards the bacillus of pertussis. Agglutination in dilution of 1-60 frequently occurred at two to three weeks after the onset of the disease. The lowest titer was 1-10 at two weeks after the onset and the highest 1-240, likewise two weeks after the onset. There was no agglutination in the serum of the patients having had pertussis eight months and eighteen months before. At the time of the onset, there was no agglutination by the serum.

About half of the cases showed a slight agglutination, 1-20, with respect to the influenza bacillus. The other half gave no agglutination with this bacillus.

CONCLUSIONS

My results indicate that complement-fixation in pertussis is obtainable about two weeks after the onset of the disease. The fixation is not as strong at this time as it is eight to ten weeks after the onset. At eight months after the beginning of the whoop, the reaction may still be present but not marked.

When the influenza bacillus is used as antigen with serum of pertussis patients, no complement-fixation occurs.

The serum of patients with pertussis, or convalescing from pertussis, agglutinates the bacillus pertussis, but the concentration of the agglutinin varies greatly.

The influenza bacillus is not agglutinated by the serum of pertussis patients in dilutions above 1-20.

These results, as well as the results of the tests for complement-fixation and agglutination with the serum of immunized rabbits, indicate no relationship between the bacillus of pertussis and the bacillus of influenza; the results with the serum of patients indicate that the bacillus pertussis has a specific relationship to whooping-cough, but that the influenza bacillus has not.

VARIOUS SPOROTRICHIA DIFFERENTIATED BY THE FERMENTATION OF CARBOHYDRATES

STUDIES ON AMERICAN SPOROTRICHOSIS, 1*

K. F. MEYER AND J. A. AIRD

(From the Department of Pathology and Bacteriology of the University of California,
Berkeley, Cal.)

Pathogenic sporotricha have been isolated during the last fourteen years in increasing numbers, and their importance as a causative agent of various forms of diseases has been fully recognized. The exhaustive studies of De Beurmann and Gougerot¹ have added valuable information to our knowledge concerning the American sporotricha. In comparing numerous organisms which they had isolated in France with other strains from North and South America, Italy, Austria, etc., assisted by the best mycologists (Matruchot and Ramond), they attempted a classification of the genus sporotrichum. Seven different species were created, to which Langeron² recently added another. The species groups discussed by De Beurmann and Gougerot are, with the exception of the sporothrix beurmanni group, represented by one or two specimens only. This fact is of considerable importance when one remembers that this group of higher fungi is so exceedingly pleomorphic, and that neither the macroscopic nor the microscopic characteristics for all the species discussed by the two French investigators vary to such a degree that, without a detailed study, a difference could be noted. The pleomorphism of *Sporothrix beurmanni* on solid carbohydrate culture media, when Sabouraud's glucose agar is not used, is so frequent, and is often so inconstant and uncontrollable, that it is not surprising to note that practically all the American mycologists (Gifford, Davis, and others), who isolated and superficially studied a small number of sporotricha, refused to accept the classification of Gougerot.

In studying carefully the publications of Gougerot, of Matruchot, and others, we became convinced however that if the facts are as

* Received for publication March 15, 1915.

1. Arch. de parasitol., 1911, 15, p. 5; Kille Wassermann: Handbuch der pathogenen Mikroorganismen, 1913, 5, p. 2.

2. Arch. de parasitol., 1913, 16, p. 307.

stated in the publications, the writers were fully justified in creating a species *Beurmanni* in addition to the species *Sporothrix schenckii*. The differences—various types of pleomorphism and pigmentation, formation of chlamydospores, fermentation of carbohydrates—are doubtless very pronounced. De Beurmann and Gougerot compared however only one strain: the so-called *sporothrix schenckii*, which was isolated by Hektoen and Perkins in 1900 and which was considered by Hektoen to be identical with the strain isolated by Schenck in 1898.

Since 1908 Hyde and Davis,⁸ Ruediger and Hiller,⁴ Page, Frothingham and Paige,⁵ Gifford,⁶ and Trimble and Shaw,⁷ have isolated in North America numerous new strains of sporotricha from human and animal cases and have shown that the "*Beurmanni*" characteristics are not always present, as far as their, in many respects, very superficial morphologic and biologic studies permit us to conclude. Davis⁸ in particular during the last two years has pointed out that there is no reason to accept the classification of De Beurmann and Gougerot as final.

His first paper dealt with the agglutination test as a means of differentiation between the various sporotricha. The sera of immunized rabbits flocculated the spores of all the different *sporothrix* strains in the same dilutions. Inasmuch as our experience has shown that the agglutination test for *sporothrix* is only applicable for clinical purposes, and not for immunological or differential tests, and that rabbits are not very suitable for immunization with sporotricha, we feel that these methods were not practicable in deciding whether *Sporothrix beurmanni* exists in North America or not. Extensive tests which were carried out by us by means of the complement fixation and agglutination tests, using horse and dog sera, only demonstrated that the different strains which we have isolated are identical from an immunological viewpoint.

In his second paper, Davis,⁹ investigating the chlamydospore production, found that the *sporothrix schenckii* also produces on special media which are poor in nutritive substances chlamydospores just as abundantly as do the European strains. We have had ample oppor-

3. Jour. Cutan. Dis., 1910, 28, p. 321.

4. Jour. Minnesota Med. Assn., 1911, 31, p. 507.

5. Jour. Med. Research, 1910, 23, p. 137.

6. Ophth. Rec., 1910, 19, p. 573.

7. Jour. Kansas Med. Soc., 1909, 9, p. 305.

8. Jour. Infect. Dis., 1913, 12, p. 140.

9. Ibid., 1914, 15, p. 580.

tunity to study the production of chlamydo-spores and have always been able to demonstrate them on ordinary Sabouraud's medium. Even the original Hektoen strain (*Sporothrix schenckii* of Gougerot) produced in our hands, in several old cultures, the spores under discussion. The strain had been passed through young rats. Some of our equine strains frequently failed, in the first two or three generations, to produce chlamydo-spores, and some of the strains still produce them in very small numbers only. On the medium of Davis, however, twelve strains selected at random produce the spores in abundance, so that we are quite prepared to confirm the statements of Davis and also to corroborate his conclusion that the presence of chlamydo-spores does not justify the identification of the strain as *Sporothrix beurmanni*.

In addition to these two points of differentiation, the pleomorphism, the pigmentation, and the fermentation properties of the sporothrix are considered in the classification of De Beurmann and Gougerot.

Pleomorphism.—The first feature has been entirely ignored in all American publications on pathogenic sporotricha. Concerning the sporothrix *schenckii* De Beurmann and Gougerot write as follows:

Unité et Pléomorphismes.—Cette étude n'est pas signalée par les auteurs américains. Les pléomorphismes des cultures étudiées (échantillon Hektoen-Gougerot) sont presque nuls. A peine note-t-on sur quelques tubes de cultures en milieux pauvres une tendance du voile à devenir lisse et sur certains tubes de pomme de terre glycosée-peptones un développement exubérant des piquants, un poudrage blanc-mat; les piquants très touffus, souvent réticulés à leur base, atteignent jusqu'à 10 et 12 millimètres de longueur et 1 à 2 millimètres de largeur; ils peuvent envahir tout le tube et s'accoller au verre en face de la culture.

En réalité, nos cultures de *Sporotrichum Schencki* (échantillon Hektoen-Gougerot) se sont montrées d'une fixité remarquable à l'inverse de celles de *Sporotrichum Beurmanni*.

The publications of Hyde and Davis,³ Hamburger,¹⁰ Trimble and Shaw,⁷ and Albert and Grover,¹¹ do not record observations of pleomorphism. Some of their strains, according to the descriptions given and according also to the pictures accompanying the articles, show clearly that it occurs also in American strains. Our observations on thirty-five strains have demonstrated that pleomorphism is a characteristic feature of most of the American strains and that it is just as common as has been described in *Sporothrix beurmanni*. On maltose agar particularly, one notices pleomorphism very regularly, just as De

10. Jour. Am. Med. Assn., 1912, 59, p. 1590.

11. Iowa Med. Jour., 1913, 19, p. 428.

Beurmann and Gougerot pointed out as early as 1906. The original strain Hektoen has, in our experience, a rather fixed growth, which can be stimulated to a pleomorphic surface growth (smooth, not folded and shiny, having powdery, dull growth) only by repeated cultivation on maltose agar.

In another publication we will discuss and illustrate this condition in detail. We will emphasize however that our strains, isolated and kept on Sabouraud's media, behave just as the Beurmanni strains with regard to the pleomorphic growth. The pleomorphic growth, if absolutely typical and constant, would indicate that the recently isolated American (human and animal) strains are of the "Beurmanni" type.

Pigmentation.—The second feature of distinction, according to De Beurmann and Gougerot, "pigmentation," is claimed to be constantly absent, particularly on potato media. The colonies remain snow-white or grayish, even when old and dry, in cultures of *Sporothrix schenckii*-Hektoen-initial strain. On the other hand, the Beurmanni strains become dark-brownish; some strains change very rapidly to a deep black. These statements of De Beurmann and Gougerot are surprising, inasmuch as Schenck and Hektoen and Perkins show clearly that a brownish, yellowish pigmentation of the old cultures also occurred; that no deeper black was observed is perhaps due to the fact that no glucose or glycerin had been added to the potatoes, as was the case in the tests of De Beurmann and Gougerot. This is not the occasion to discuss the possibility that the original Hektoen strain, by growth on improper media during the six years since its isolation, had been entirely modified. In our hands the Hektoen strain formed pigment on glycerin-peptonized potatoes, so that it could be classified with the B-Beurmanni strains.

The recently isolated strains of Davis, Hamburger, Trimble and Shaw, Gifford, and Page, Frothingham and Paige, all show distinct pigmentation. Trimble and Shaw⁷ report, for example, that their cultures showed a "turning brown, deepening to black with age." The plate accompanying the publication of Page, Frothingham and Paige⁸ shows a most decided blackening of the potato cultures, so that one would not hesitate to diagnose the strain, from these drawings, as a type of *Sporothrix beurmanni*. All these facts make it apparent that the pigmentation is not always absent in the "Schenckii strains," and in the light of the work of De Beurmann and Gougerot, the bac-

teriologists who have isolated sporotricha in this country since 1910 could not safely diagnose their strains as *Sporothrix schenckii*. The statement of Gifford (which, according to our experience, is correct) that an American pigmented sporothrix strain could be turned to an unpigmented Schenckii-like strain when grown on lactose agar, or influenced by other means, does not affect the question of pigmentation on the differential media like glucose agar and potato, and it would be better if such attempts of mutation were not used to complicate the already existing difficulties of classification.

Strains of the α , β , and γ type, as recognized and clearly defined by De Beurmann and Gougerot, have been isolated by us, as will be shown later. However, the pleomorphism of pigmentation is not absolutely constant, and as soon as one studies a large number of strains, one will agree with De Beurmann and Gougerot, who state that an α strain can be easily changed into a β or γ strain, and vice versa. This characteristic cannot be used therefore for differentiation.

Fermentation of Carbohydrates.—The third feature, the fermentation of carbohydrates, is constantly reported by De Beurmann and Gougerot as being a distinctive phenomenon between *Sporothrix schenckii* and *Sporothrix beurmanni*. Even when one is confronted with the fact that the method of biochemical tests in these groups of organisms is not as easy and quick a procedure as it is in the colon-paratyphoid group, a comparative study to verify the statements of De Beurmann and Gougerot suggests itself.

Gougerot and Blanchetière¹² reported that all the Beurmanni strains studied by them fermented saccharose but not lactose; the original Hektoen strain of *Sporothrix schenckii* hydrolyses and ferments lactose, but not saccharose. Schenck¹³ and Hektoen and Perkins¹⁴ report only that in the fermentation-tubes no gas is liberated by the growth of the sporotricha in lactose, glucose, and saccharose broth; there are no statements as to the acid production. Page, Frothingham and Paige⁵ report that their two equine strains produced only acid in glucose, galactose, and dextrin serum-water-litmus media; but as to the last two carbohydrates, they desire further confirmation.

In 1913 a preliminary test was carried out by one of us (K. F. M.), using a somewhat modified medium from the one recommended by Blanchetière and Gougerot, and selecting from our collection of 35

12. Compt. rend. Soc. de biol., 1909, 16, p. 202.

13. Bull. Johns Hopkins Hosp., 1893, 9, p. 286.

14. Jour. Exper. Med., 1909, 5, p. 77.

strains, 15 strains which showed, on Sabouraud's media, more or less striking differences.

The strains were all grown in small Erlenmeyer flasks (10 c.c.) for sixty to ninety days, and by means of cork floaters a surface growth was obtained. Only those flasks on which a good, thick, felt-like surface growth was present, were considered suitable for a final reading. As an indicator, only litmus was used. The cultures were kept in a cupboard, in the space situated between the double doors of an incubator-room; the temperature varied from 26-32 C. On account of the number of flasks and the lack of available space, only seven carbohydrates—glucose, lactose, maltose, saccharose, mannite, galactose, levulose, and glycerin—were tested. Only Merck purest chemicals were used. The results are compiled in Table 1.

All strains fermented decidedly glucose with acid production but no gas. No strain fermented lactose or mannite. This result is surprising, inasmuch as the *sporothrix schenckii* should ferment the lactose. With regard to the other carbohydrates, no uniform results were obtained. Most of the strains, with the exception of an atypical one, fermented levulose; only seven fermented saccharose; maltose was inverted by only five strains; galactose was changed by practically the same strains. As far as tested, glycerin was fermented in the same irregular manner as noted for the other strains. These results confirm the statements of Blanchetière and Gougerot, that pathogenic sporotricha—particularly *Sporothrix beurmanni*—ferment glucose, maltose, saccharose, galactose, levulose and glycerin, but not lactose and mannite. However, in our series less than 40 percent of the strains fermented the carbohydrates mentioned in a regular manner; most of the strains attacked only glucose and levulose. The strains fermenting saccharose, therefore, would be classified according to Blanchetière and Gougerot with the *sporothrix beurmanni*. These developed marked fermentative activities also in the other carbohydrates. Unfortunately, the test strain *Sporothrix beurmanni* obtained from D. J. Davis failed to give the reactions claimed by Blanchetière and Gougerot.

The amount of acid produced was not tested in detail, but it varied considerably. At the first glance it is apparent that those strains which were perpetuated for several generations on Sabouraud's medium fermented more freely the different carbohydrates than the more recently isolated ones. This point, however, is not constant. Some strains like B and U are decided *Sporothrix beurmanni* strains in their morphological appearance and growth on the various test media. Strain B was identified by Dr. de Beurmann, in a personal communication to one of us, as a *sporotrichum beurmanni*. This superficial test shows only that some *sporothrix* strains will ferment various carbohydrates; the results, however, are irregular, inconstant, and of no diagnostic value. The strain T proved, on further examination, to be a blastomycotic sporotrichum, as first suspected.

In 1914, after most of the strains had been kept on Sabouraud's medium for many months, it was thought best to test again the fermentative action of a series of sporotricha strains, selected at random, growing the same for a longer period and in large (1 L. and 500 c.c.) flasks. The cultures were kept at room temperature, without tin-foil or rubber-cap covering of the cotton plugs. Furthermore, it was considered advisable to use the medium recommended for such tests by Blanchetière. Also, a strain identified by the Institut Pasteur as *Sporothrix beurmanni* was used as a control. The acid production was determined by titration with N/20 NAOH after 68, 202, and 270 days, respec-

TABLE 1
FERMENTATION BY *SPOROTRICHA*

Carbohydrate, 4 percent; litmus, 5 percent; peptone, 1 percent solution.

Strains *	Glucose	Lactose	Saccharose	Maltose	Mannite	Galactose	Levulose	Glycerin
<i>Sp. schenckii</i> (D. J. Davis) (Hektoen-initial) Hektoen-Gaucherot.....	+	0	0	0	0	0	+	+
<i>Sp. Beurmanni</i> (D. J. Davis) (French strain).....	+	0	0	0	0	0	+	+
Strain A/7.....	+	0	0	0	0	0	+	+
Strain AB/4.....	+	0	0	0	0	0	+	+
Strain B/8.....	+	0	0	0	0	0	+	+
Strain C/7.....	+	0	0	0	0	0	+	+
Strain D/5.....	+	0	0	0	0	0	+	+
Strain E/5.....	+	0	0	0	0	0	+	+
Strain F/4.....	+	0	0	0	0	0	+	+
Strain G/4.....	+	0	0	0	0	0	+	+
Strain K.....	+	0	0	0	0	0	+	+
Strain L.....	+	0	0	0	0	0	+	+
Strain M.....	+	0	0	0	0	0	+	+
Strain T.....	+	0	0	0	0	0	+	+
Strain U/4.....	+	0	0	0	0	0	+	+

* The history of these strains is given in the appendix.

The sign + + + means decidedly acid, bright red; + + acid, purplish; 0 unchanged; — not tested. The figures indicate the acidity determined by titration.

tively, of cultivation. Only four carbohydrates (Merck's chemicals), glucose, lactose, saccharose, and starch, were used.

The results are tabulated in Table 2. The figures indicate the actual amount of acid produced after having deducted the figure indicating the acidity in the control flasks.

TABLE 2
FERMENTATION BY SPOROTRICHIA

Strains	Lactose		Saccharose		Glucose	Starch
	68 Days	270 Days	68 Days	270 Days	202 Days	202 Days
Sp. beurmanni.....	0.11	.6	0.15	0.8	4.35	4.35
Sp. hamburger.....	.00	0.5	0.14	0.4	4.25	1.05
Sp. schenckii (Hektoen- Gougerot) (Hektoen- initial).....	.00	0.25	0.20	0.45	4.55	1.05
B 12.....	.00	0.35	0.84	3.0	2.65	2.95
C 10.....	.00	0.1	0	0.15	2.50	3.95
F 6.....	0.13	0.4	0.28	4.05	3.20	0.65
K 7.....	0.35	0.6	0.58	2.6	2.20	0.8
L 7.....	0.13	0.45	0.08	0.35	3.15	2.05
Z 6.....	0.10	0.3	0.13	3.4	4.60	1.95
DD 5.....	0.12	0.55	0.27	3.4	3.1	2.7
AB 10.....	.00	0.55	0.20	2.9	3.2	0.55
CC 4.....	0.03	..	0.08	3.45	3.8	1.4

The results show that all strains (12) tested, decidedly ferment glucose. The acid produced was, as previous tests had shown, in the main lactic acid. Lactose was not attacked by the strains after sixty-eight days' cultivation; after 270 days, however, every strain produced traces of acid. The nature of it has not as yet been determined, but it appears from other tests that similar small amounts of acid are produced by sporotricha in media not containing carbohydrates; this fact is observed in similar tests with other organisms (typhoid, anaerobes, etc.). It is therefore apparent that the sporothrix schenckii strains (Sporothrix Hamburger and Schenckii—original—Hektoen) did not ferment lactose. These findings are in contradiction to those of Blanchetière and Gougerot. The fermentation of saccharose and starch was irregular. Only seven strains fermented saccharose, the control strain of Sporothrix beurmanni failing to produce a marked acidity which could be considered as a fermentation of the carbohydrates. Following the classification of Blanchetière and Gougerot, the strains B₁₂, F₆, K₇, Z₆, DD₅, AB₁₀, and CC₄, would be true Sporothrix beurmanni strains. The acid production in starch is not marked for two strains only; the growth in these flasks was poor compared with the other cultures. The absence of hydrolysis in these flasks and the subsequent absence of glucose explains, in our opinion, the dis-

crepancies. Blanchetière and Gougerot found fermentation of starch to occur for both *Sporothrix beurmanni* and *schenckii*.

In considering these results purely from a differential diagnostic viewpoint, it is quite evident that it cannot be used for this purpose, and the fermentation of carbohydrates is just as little a criterion of the type of sporotrichum as is the absence of pleomorphism and the chlamydospore formation.

Blanchetière and Gougerot tested only six varieties of sporotricha in saccharose, of which only four fermented this carbohydrate. The report gives no information as to the classification of the varieties. It is mentioned however that *Sporothrix schenckii* was one of the two varieties which did not ferment saccharose.

These two investigators expressed the opinion that they were unable to state whether the fermentative reactions were entirely fixed for all the Beurmanni strains, or whether, on account of the tendency to pleomorphism, certain variations were possible. The findings of Greco,¹⁵ with a strain which he named "*Sporothrix schenckii-beurmanni*," isolated in Uruguay, are similar to ours and therefore prove conclusively that the fermentative activities of the sporotricha are not fixed. The strain of Greco did not ferment mannite, lactose, and saccharose.

During the course of these observations it was noted that the fermentative activities showed some relation to the growth of the strains on Sabouraud's medium. The amount of pigment, and the time of its appearance, correspond in some respects to the ability to ferment saccharose.

De Beurmann and Gougerot distinguished, according to the pigmentation on Sabouraud's medium, a sporotrichum α , β , and γ . The type " α " is, ordinarily, very poorly pigmented; it remains white or light-brownish; the pigment appears slowly, and only portions or segments of the colonies are tinged. The " β " type reaches a chocolate-brownish color and pigments slowly also. The " γ " type changes from a dirty gray color to a deep ebony black in four to five days. The tinge is always deep black, or brownish-black, with brownish or steel-bluish reflections. The distinction of these three types is not absolute and cannot be used for differentiation. In our experience, however, the ability to form pigment remained fairly constant for the strains studied by us (with one exception) for the period of four years during which we made observations. The classification of the various sporo-

15. Argentina Med., 1907, p. 699.

tricha together with their acid production in saccharose, is shown in Table 3.

TABLE 3
CLASSIFICATION AND ACID PRODUCTION OF SPOROTRICHIA

		68 Days	270 Days
"α" type..	{ Strain B	0.84	3.0
	{ Strain F	0.28	4.05
	{ Strain Z	0.13	3.4
	{ Strain AB	0.20	2.9
"β" type..	{ Strain K	0.58	2.6
	{ Strain CC	0.08	3.45
	{ Strain DD	0.27	2.4
"γ" type..	{ Strain C	0.0	0.15
	{ Strain L	0.8	0.35

The strains producing only small amounts of pigment proved to be the most vigorous saccharose fermenters. This fact is proven in a very interesting observation. Strain C fermented, according to Table 1, saccharose. In the last test, however, it failed to produce a marked amount of lactic acid. According to our records the strain was a β type when isolated; passed, later, through several animals, it changed entirely to a γ type. The original culture having been lost, one of the subcultures of the animal-passage was used without further consideration. The result is clearly shown; the strain does not ferment saccharose in the second test. In our opinion this observation is further proof that the carbohydrate fermentation is inconstant and dependent upon numerous factors, of which animal passage seems to be one. The strains of Page, Frothingham and Paige being of the γ type behaved therefore correctly in not fermenting the saccharose.

For the existing relation of pigmentation to fermentation, we are at present unable to offer any definite explanation. Some additional experiments are in progress which we hope will throw some light on this phase of the chemical activity of the sporotricha.

CONCLUSIONS

The differentiation of pathogenic sporotricha into two distinct species, by means of the fermentation of carbohydrates, is impossible. The reactions are not fixed and are as inconstant as the many variations noted in the formation of chlamydo-spores and, frequently, in pleomorphism. There does exist however an apparent relation between the pigmentation of the sporotrichum strains and the ability of these strains to ferment saccharose. The α and β types are the most active fermenters.

This and other evidence, which will be presented elsewhere, make it apparent that the American sporotricha—of which we studied thirty-five strains—have, in many respects, type characters in common with *Sporothrix beurmanni*. In the light of De Beurmann's and Gougerot's

work, some of the American strains are doubtless *Sporothrix beurmanni*, and it is not permissible to call such strains "*Sporothrix schenckii*" merely for the sake of simplicity. The discussion of De Beurmann and Gougerot¹⁶ on this subject can now also, in our opinion, be satisfactorily closed, namely: that *Sporothrix schenckii*, Hektoen-Gougerot strain, is an absolutely fixed type. The true *sporothrix schenckii* is represented however by all of the recently isolated strains. Inasmuch as most of these strains are undoubtedly identical with *Sporothrix beurmanni*, the *sporothrix schenckii* is identical with the *sporothrix beurmanni*.

The American strains of pathogenic sporotricha are therefore best classified as one species: *Sporothrix schenckii-beurmanni* (as suggested by Greco).

APPENDIX

HISTORY OF THE STRAINS

Sp. *beurmanni*: Original tube was received from Institut Pasteur, Paris, March, 1914.

Sp. *schencki*: Original tube was received from Dr. D. J. Davis, Chicago, 1913 and 1914. This is the so-called "Hektoen-Gougerot-initial" strain.

Sp. *schenckii* (Hamburger): Original tube was received from Dr. D. J. Davis, Chicago.

Strain "A": Original was isolated by K. F. Meyer from pus collected from a horse with so-called epizootic lymphangitis, October, 1911.

Strain "AB": Original was isolated by K. F. Meyer, October, 1911, from experimental horse, "Nancy," January, 1912.

Strain "B-12": Original was isolated by K. F. Meyer from pus collected from a horse with so-called epizootic lymphangitis, April 2, 1912.

Strain "C-10": Ditto; by K. F. Meyer; ditto, May 23, 1912.

Strain "CC": Original was isolated from a human case of sporotrichosis, April 30, 1913, by K. F. Meyer.

Strain "D-5": Original tube was received from Dr. D. J. Davis, Chicago, December, 1911.

Strain "DD-5": Original was isolated by K. F. Meyer from equine case of sporotrichosis, June 4, 1913.

Strain "E-5": Original was isolated by K. F. Meyer, collected from a horse with so-called epizootic lymphangitis, July, 1912.

Strain "F-6": Ditto; July 1, 1912.

Strain "G-4": Original tube was received from Dr. G. F. Ruediger, North Dakota, July, 1912.

Strain "K-7": Original was isolated by K. F. Meyer from a human case of sporotrichosis in Strattonville, Pa., July 1, 1912.

Strain "L-7": Original was isolated by K. F. Meyer from an equine case of sporotrichosis July 19, 1912.

Strain "M": Original was isolated by K. F. Meyer from pus collected from a mule with so-called epizootic lymphangitis, January 3, 1913.

Strain "T": Original, a blastomycotic strain of *sporothrix*, was isolated by K. F. Meyer from an experimental case of sporotrichosis, August, 1912.

Strain "Z-6": Original was isolated by K. F. Meyer from an equine case of sporotrichosis, April 14, 1913.

16. Bull. et mém. Soc. Med. d. hôp. de Paris, 1908, 26, p. 9.

INDIVIDUAL AND GROUP VARIATION IN GUINEA-PIGS IN THE AMERICAN METHOD OF TESTING TETANUS ANTITOXIN *

LOREN B. TABER

(*The Cutter Biological Laboratories, Berkeley, California*)

At times during the testing of the potency of tetanus antitoxins by the American method,¹ due to the overestimation of unitage, the entire series of guinea-pigs on the test of a serum dies in less than ninety-six hours, which is the official test period.² Thus the potency is left undetermined. It then becomes necessary to put on another series of guinea-pigs receiving, with the test dose of standard toxin, lower dilutions of the serum.

In such cases it would be of advantage to be able to estimate, from the first series, the dilution of antitoxin necessary to add to the test dose of toxin in order to indicate the potency of the antitoxin in standard units. Ability to do this would result in the saving of both guinea-pigs and labor.

During the one and one-half years in which I have been engaged in testing the antitetanic sera in this laboratory, we have had occasion to test an extensive line of sera. For the most part, these were from freshly drawn blood, but there were a number also which were tested after having stood for varying periods under different conditions, such as storage in a cool, dark vault in a large, amber bottle, or storage on the shelves of a drug-store in the syringe container. Some were filtered before testing, others not. In short, the testing was carried out with the same kinds of sera and under the same conditions that obtain in any commercial laboratory. Variation due to the personal equation being eliminated, an analysis of the test records for this period, chosen otherwise according to their acceptability as experimental data, would reveal information of interest.

A preliminary examination of the balance, the buret, and the graduated pipettes used in measuring the test materials, showed that any error introduced through their inaccuracies would not be worthy of consideration.

* Received for publication, March 15, 1915.

1. Bull. Hyg. Lab., U. S. P. H. and M.-H. S., 1907, 43.

2. *Ibid.*, p. 6.

METHOD OF ANALYSIS

The test animals are considered in pairs. An injection was made into the tissues of the abdomen of each individual of the pair about the level of the umbilicus at the same time, with the same volume (4 c.c.) of a toxin-antitoxin mixture in 0.85 percent NaCl solution, which had stood at room temperature for one hour in diffuse light, and which had been prepared from the same toxin and antitoxin dilutions. In each toxin-antitoxin mixture injected, there was exactly the test dose of standard precipitated toxin (United States standard tetanus toxin "D") together with a volume of antitoxin which varied with the test animal. The animals were selected from similar weight-groups—260-300 gm., 300-340 gm., 340-360 gm. (the official weight-group for testing antitetanic sera), and 360-400 gm. Only pairs from the same weight-groups were considered together. It sometimes happens that the data pertaining to one individual guinea-pig is used in more than one pair. This is permissible when there are three or more guinea-pigs in the same series, the other conditions being fulfilled.

In Table 1, an example of the method of assembling the fundamental data is given. There were in all 167 different tests, comprising 401 pairs of pigs made up from 443 different animals.

The fundamental data having been assembled, the process of analysis was begun by segregating the pairs of guinea-pigs according to the time elapsing before the death or symptoms of the guinea-pig receiving the smaller volume of antitoxin, as follows:

A. Pig receiving smaller volume of antitoxin died in 0-47 hours following injection.

B. Pig receiving smaller volume of antitoxin died in 48-71 hours following injection.

C. Pig receiving smaller volume of antitoxin died in 72-95 hours following injection.

D. Pig receiving smaller volume of antitoxin died in 84-108 hours following injection.

E. Pig receiving smaller volume of antitoxin died in 96-119 hours following injection.

F. Pig receiving smaller volume of antitoxin died in 120-143 hours following injection.

G. Pig receiving smaller volume of antitoxin died in 144-240 hours following injection.

H. Pig receiving smaller volume of antitoxin showed fatal symptoms but did not die in less than 120 hours. (Sometimes observations were not made after the fifth day.)

I. Pig receiving smaller volume of antitoxin showed non-fatal symptoms, or no symptoms.

TABLE 1

AN EXAMPLE OF THE METHOD OF ASSEMBLING THE FUNDAMENTAL DATA

Test Number of Each Series of Animals Put on the Same Serum at the Same Time	Number of Serum	Date of Test	Weight of Guinea-pigs Taken in Groups in Grams	Smaller Volume of Antitoxin Received by First Guinea-pig	Time Elapsing Before Death or Symptoms Due to Smaller Volume	Larger Volume Received by Second Guinea-pig	Time Elapsing Before Death or Symptoms Due to Larger Volume	Percentage Depense of Smaller Vol. of Antitoxin from Larger Volume
1	Serial 217	9/30/14	300-340	$\left\{ \begin{array}{l} 1 \\ 3333 \end{array} \right\}$	1* 36 (—) hr.	1	† 51 hr.	20
				$\left\{ \begin{array}{l} 1 \\ 3333 \end{array} \right\}$	† 33 (—) hr.	$\left\{ \begin{array}{l} 1 \\ 2222 \end{array} \right\}$	† 77 hr.	34
				$\left\{ \begin{array}{l} 1 \\ 2666 \end{array} \right\}$	† 51 hr.	$\left\{ \begin{array}{l} 1 \\ 2222 \end{array} \right\}$	† 72 hr.	16
81	Op. 96	9/10/14	340-360	$\left\{ \begin{array}{l} 1 \\ 2000 \end{array} \right\}$	†* 60 (†) hr.	1	† 240 hr.	37
				$\left\{ \begin{array}{l} 1 \\ 2000 \end{array} \right\}$	† 60 (†) hr.	$\left\{ \begin{array}{l} 1 \\ 741 \end{array} \right\}$	*	63
				$\left\{ \begin{array}{l} 1 \\ 1250 \end{array} \right\}$	† 240 hr.	$\left\{ \begin{array}{l} 1 \\ 741 \end{array} \right\}$	—	40
57	Op. 88	7/25/14	260-300	$\left\{ \begin{array}{l} 1 \\ 500 \end{array} \right\}$	† 96 hr.	$\left\{ \begin{array}{l} 1 \\ 303 \end{array} \right\}$	b,* 216 hr.	30

* Symbols; + = death in number of hours indicated; b = symptoms indicate non-fatal issue; (—) = less than time indicated by not more than seven hours; (†) = more than time indicated by not more than seven hours; — = no symptoms caused by infection.

† This percentage difference between the denominators of the two fractional volumes of sera is in terms of the denominator of the smaller volume.

In these tabulations the second guinea-pigs of the pairs, i. e., the pigs receiving the larger volume of antitoxin, were distributed in horizontal columns according to their death periods or symptoms, as in the case of the first guinea-pigs, and in vertical columns according to the percentage-decrease in unitage at which they were tested. Arbitrary groupings of these percentage-decreases were made, for the sake of convenience. Only pairs belonging to the same weight groups were considered together.

This analytical scheme should at once reveal any constant relation between any two variable factors, the rest of the variables being constant so far as experimentally practicable. As a matter of fact, it is seen at a glance that a broad relation between the percentage-decrease of unitage tested for in the second guinea-pig, and the time elapsing before death or the symptoms of the first guinea-pig, does exist as indicated by the time elapsing before death or the symptoms of the second guinea-pig. The larger percentages are grouped about central points, but there are so many irregularities of such striking variation from the central type, that these relations cannot from this tabulation be expressed in terms of numerical frequencies which would have any practical value. It is also apparent that the resistance to the unbound toxin offered by members of different weight-groups varies, but does not vary constantly. Sometimes the heavier guinea-pigs show the greatest resistance, at other times the lighter guinea-pigs possess this faculty to greater degree; occasionally there is concordance in this respect between all weights considered.

In order to attain the practical results for which we are striving, it is necessary to condense the tabulations just considered. This is done in Table 2, all guinea-pigs receiving the larger amount of antitoxin being classed in one of the four groups following:

1. Died in 0-95 hours.
2. Died in 96-119 hours.
3. Died in 120 or more hours, or showed fatal symptoms.
4. Did not show fatal symptoms.

From this condensed tabulation the group relations are now more apparent. Individual variations among the guinea-pigs, however, are still the cause of considerable discrepancies, especially in those cases in which, in the group under observation, only a few animals are included. Examination of the relations existing between the comparative resistances of the different weight-groups to unbound toxin

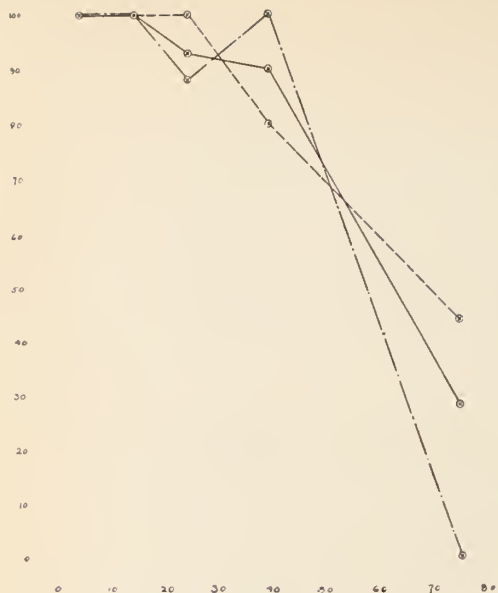


Chart 1.—Pig receiving smaller volume of antitoxin died in 0-47 hours.



Chart 2.—Pig receiving smaller volume of antitoxin died in 47-71 hours.

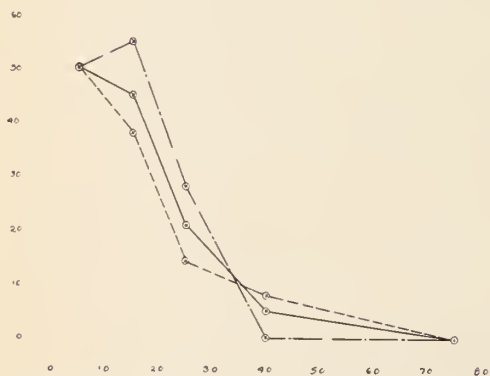


Chart 3.—Pig receiving smaller volume of antitoxin died in 72-95 hours.

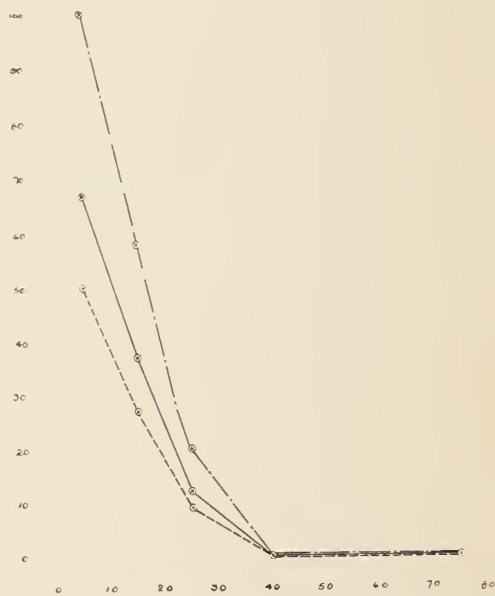


Chart 4.—Pig receiving smaller volume of antitoxin died in 84-108 hours.

COMPARATIVE RESISTANCES OF DIFFERENT WEIGHT GROUPS OF GUINEA-PIGS TO THE SAME TOXIN-ANTITOXIN MIXTURE

Ordinates represent percentage of pigs receiving the larger volume of antitoxin dying in less than 96 hours; abscissae, the percentage decrease groups of pigs receiving larger volume of antitoxin.

Long broken lines represent weight group 340-360 gm.; short broken lines, the weight group 300-340 gm.; and solid lines, the weight group 300-360 gm.

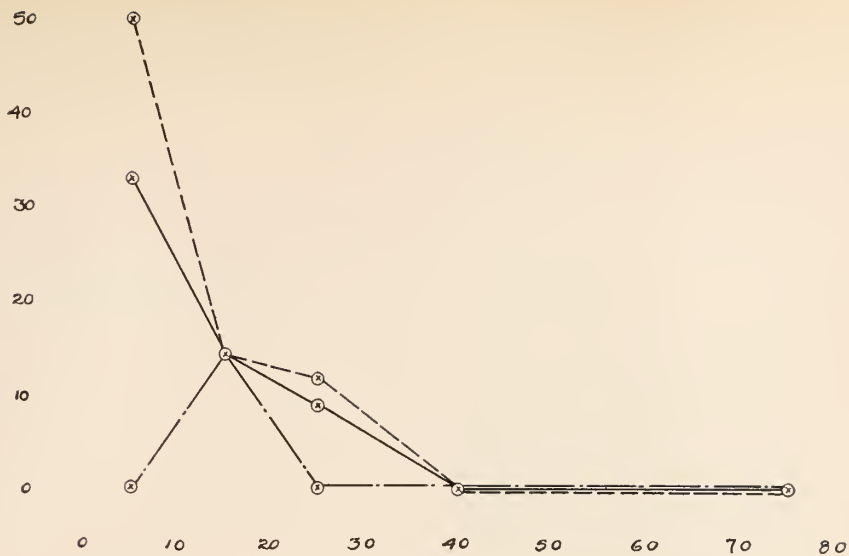


Chart 5.—Pig receiving smaller volume of antitoxin died in 96-119 hours.

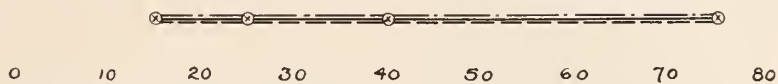


Chart 6.—Pig receiving smaller volume of antitoxin died in 120-143 hours.

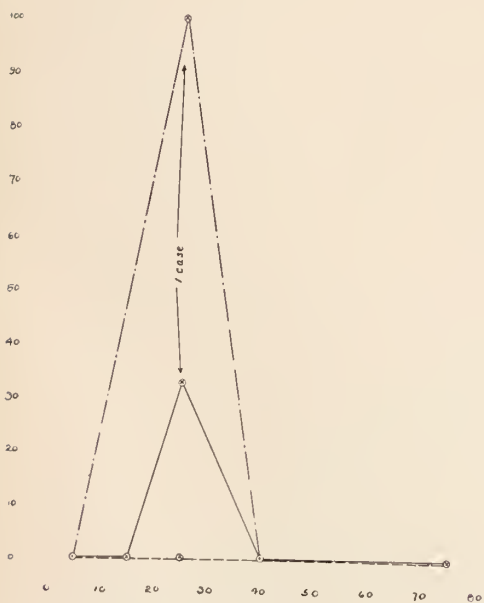


Chart 7.—Pig receiving smaller volume of antitoxin died in 144-240 hours.



Chart 8.—Pig receiving smaller volume of antitoxin did not die in less than 120 hours, but showed fatal symptoms.

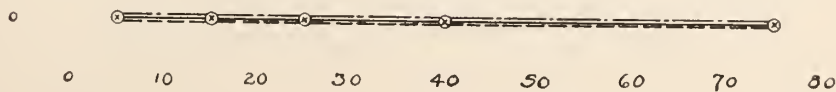


Chart 9.—Pig receiving smaller amount of antitoxin showed non-fatal or no symptoms.

under otherwise similar conditions fails to show any constancy, sometimes the lighter, sometimes the heavier being superior in this respect. Adequate comparison was impossible in the cases of weight groups 260-300 gm. and 360-400 gm., on account of numerical insufficiency, so that they are not considered in this analysis any further. The lack of constancy was however well evinced in the groups 300-340 gm., and 340-360 gm., and for this reason it was deemed expedient to form, for the sake of comparison, a third group including both of these groups, i. e., including all guinea-pigs weighing from 300-360 gm. This was done, with the result that the group relations were made still clearer (Table 2).

In order to present these figures in such form as to make their relations evident at a glance, a selected series was plotted in curves (Charts 1-9).

Chart 1 represents all pairs of guinea-pigs in which the guinea-pig receiving the smaller amount of antitoxin died in 0-47 hours; Chart 2 represents all pairs of guinea-pigs in which the guinea-pig receiving the smaller amount of antitoxin died in 48-71 hours; and so on. The abscissae represent the percentage-decrease groups for pigs receiving the larger volume of antitoxin; the ordinates, the percentage of pigs within those groups, dying in less than ninety-six hours. Different kinds of lines represent the curves for the weight groups now under consideration, viz., 300-340 gm., 340-360 gm., and 300-360 gm. Excessive irregularities due to single variations are noted on the charts.

Group relations now stand out quite clearly. It is at once evident that within the limits indicated by these curves, there is a close parallelism between the resistance of the weight groups 300-340 gm., and 340-360 gm., to the same quantity of unbound toxin. Their average curve, that of weight group 300-360, is quite smooth; more so, in fact, than one would anticipate from a survey of the figures given in the preceding tables. It is also evident, as is shown in Table 2, that the reaction of a group of guinea-pigs to the injection of a toxin-antitoxin mixture, containing several times the M. L. D. of unbound toxin, is more constant than the reaction of a similar group of guinea-pigs to the injection of a similar mixture, containing just one fatal dose, or slightly more, of unbound toxin. This supports the superiority of the ninety-six-hour death period over any one which would involve the use of a lesser amount of unbound toxin.

TABLE 2.—SEGREGATION OF PIGS RECEIVING SMALLER AMOUNT OF ANTITOXIN INTO HORIZONTAL SECTIONS, ACCORDING TO THEIR DEATH PERIOD OR SYMPTOMS. SEGREGATION OF THE OTHER PIG OF THE PAIR WITHIN THESE SECTIONS ACCORDING TO (1) PERCENTAGE-DECREASE OF UNITAGE INDICATING DOSE; (2) DEATH-PERIOD OR SYMPTOMS, (3) WEIGHT-GROUPS

[illegible]

under otherwise
times the lighte
Adequate comp
260-300 gm. a
so that they ar
of constancy v₃
340-360 gm., =
for the sake of
groups, i. e.—
This was de s₃
still clearer —

In order—
relations ev
(Charts 1-5)

Chart 1—
receiving t₁—
represents $\frac{0}{0}$
the smaller
abscissae —
the larger $\frac{0}{0}$
within th₀
kinds of —
consider $\frac{0}{0}$
sive irre₀

Grou₀
that wit₀
ism bet₀
340-360₀
curve, $\frac{0}{0}$
fact, t₀
the pr₀
the re
toxin
toxin
pigs
dose
ority or₀ ...
involve the use

DOSAGE ESTIMATION

The practical value of the foregoing discussion may be found in Table 3, which shows dosage estimations.

TABLE 3
ESTIMATION OF DOSAGE FROM DEATH PERIOD OF GUINEA-PIG DYING IN LESS THAN
NINETY-SIX HOURS

Time Elapsing Before Death of Pigs Receiving Smaller Volume of Antitoxin	Pigs Receiving Larger Volumes of Antitoxin		
	Percentage- Decrease of Units Indicated	Percentage- Dying in Less Than 96 Hours	Percentage-Decrease at Which They Should Be Tested to Give Unitage-Indicating Symptoms
0-47 hours	30-49	90	Considerably more than 50 percent
48-71 hours	20-29 30-49	53 17	35 percent (more or less according to time of death of first guinea- pig)
72-95 hours	10-19 20-29 30-49	35 21 5	25 percent (more or less according to time of death of first guinea- pig)

INQUIRY INTO THE ACCURACY OF THE AMERICAN METHOD

I was now led to inquire into the accuracy of the American method of testing tetanus antitoxins. To determine this I analyzed the results obtained from increasing the dose for guinea-pigs dying in 72-95 hours, 84-108 hours, and 96-119 hours (Table 2). Interpolating the values given in these tables, we obtain the following figures:

TABLE 4
LIMITS OF ACCURACY OF THE AMERICAN METHOD

Time of Death of Guinea-pig Receiving Smaller amount of Antitoxin	Percentage- Decrease of Unitage Indicating Dose	Percentage of Guinea-pigs Receiving Larger Volume Dying in Less Than 96 Hours
72- 95 hr.	21	25
84-108 hr.	19	25
96-119 hr.	9	25

It is certainly true that any method of testing biological products should work in at least 75 percent of cases. If this arbitrary standard (which is deliberately made low, so as to place the method in as favorable light as possible) is accepted, we can then say that the American method of testing antitetanic sera cannot be depended upon to be accurate within less than approximately 15 percent of the theoretical unitage. In no case should the doses be spaced more closely than this limit.

GROUP VARIATION

It should furthermore be kept in mind that individual guinea-pigs are susceptible to variations in resistance which are at times surprising, and that apart from individual variations, groups of guinea-pigs from different sources, or groups kept under different conditions, will show variations as a whole when tested against the same sera with the same toxin. Typical examples of individual, and possibly also of group, variation are shown in Table 5. In the two tests made on Nov. 11, 1914, five guinea-pigs out of six show a greater resistance than is shown by guinea-pigs on corresponding doses in previous tests. This may be, of course, only a chance alignment of individual variations, such as is always liable to happen in a short series. Actual proof of the existence of group variation will be considered at more length later.

If the approximate potency only were desired, expressing the unitage of either of the sera tested in the foregoing table by any one of the numbers given for it under the column "indicated unitage" would be perfectly fair. Manufacturers of antitetanic sera for commercial use in the United States, however, have to meet commercial conditions, i. e., while complying with the government regulations under which antitetanic sera must be marketed, they must also meet competition. These factors compel them (apart from their interest in the problem from the viewpoint of pure science) to inquire into the accuracy of the standard methods of testing biologics, so that their products may be graded sufficiently above the required standard to insure them a faultless reputation for quality; and at the same time, the ever present necessity in competitive manufacturing for decreased cost of production demands that this excess over the required standard shall not be great enough to put them in the rear of their competitors, other conditions being equal.

In order to be in a position in which he shall know just how much to put in a container which is guaranteed to contain a minimum number of units of antitetanic serum up to a certain "return date," which is stamped on the package, the manufacturer must first determine, with reasonable accuracy, the indicated unitage of the serum which he intends to use, and then he must allow over that figure a factor of safety sufficiently large to more than counterbalance any error which

TABLE 5

ILLUSTRATING VARIATIONS IN RESISTANCE OF GUINEA-PIGS OF DIFFERENT GROUPS TESTED AT DIFFERENT TIMES TO THE SAME TOXIN-ANTITOXIN MIXTURE

Serum Number	Test-Number	Date of Injection	Toxin	Anti-toxin in c.c.	Weight of Guinea-pig in gm.	Time of Death or Symptoms	Indicated Unitage
102	38	10/22/14	.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{8000}$	375	? ad+ death in 92 hr.	800—
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{6154}$	380	— — eddd+ death in 156 hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{4000}$	377	— — acddd released	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{2000}$	405	— — aaaah released	
102	39	11/19/14	.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{9090}$	325	? hd+ death in less than 84 hr.	800
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{8000}$	320	? hed+ death in less than 108 hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{7000}$	350	— — abedd+ death in less than 170 hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{6024}$	330	— — becdddd+ death in less than 240 hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{5000}$	330	— — — ahahhhbbba released	
103	40	11/ 6/14	.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{8333}$	400	— d+ death in 66 hr.	600? 400?
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{7000}$	340	— ed+ death in 72 hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{6024}$	350	— hdd+ death in 108 (—) hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{5000}$	355	— hd+ death in 84 (+) hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{4000}$	345	— bed+ death in 108 (+) hr.	
103	41	11/19/14	.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{6024}$	322	— — d+ death in 84 (—) hr.	500
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{5000}$	345	— ? hdd+ death in 132 (—) hr.	
			.0006 gm. U. S. A. "D" in 1 c.c. 0.85 percent NaCl	$\frac{1}{4000}$	308	— ? beddd+ death in 180 hr.	

— = no symptoms.

? = extremely slight or no symptoms.

a = slight symptoms.

h = symptoms more severe (guinea-pig easily regains feet when placed on back).

c = severe symptoms (guinea-pig when placed on back regains feet only with great difficulty).

d = very severe symptoms (guinea-pig when placed on back cannot regain feet).

+ = death.

(—) = death in not less than 7 hr. of time indicated.

(+) = death in not more than 7 hr. of time indicated.

may arise due to natural variation in testing, together with any decrease in the potency of the serum, which he anticipates might occur while the container is being kept, previous to the "return date," under the not too ideal conditions of the drug store.

The custom of this laboratory, confirmed by experience, has been to allow 25 percent for this factor of safety. We have yet to find a sample of "returned" tetanus antitoxin failing to test well within this margin.

ANALYSIS OF TABLE 2 OF BULLETIN 43

In the light of the conclusions derived from the foregoing analysis, it would be of interest to examine the results obtained in the series of tests given on pages 9 and 10 of Bulletin 43 of the Hygienic Laboratory, Washington (Table 2 of the Bulletin). In this series of forty-two guinea-pigs, each pig receives the L + dose of toxin plus just that quantity of antitoxin which should save its life for ninety-six hours. The death periods show however considerable variation, even with guinea-pigs in the same weight group put on test simultaneously. In Table 6, I have given a summary of average weights of these guinea-pigs, together with their mean death periods, average deviation of death periods from the mean, and from the standard, death period (ninety-six hours), and the greatest deviation of death period from the mean and from the standard. These are given for the weight groups 280-300 gm., 300-340 gm., 340-360 gm., 360-380 gm., so as to parallel the method of analysis of the data given in the first part of this paper. The time between the extremes of variation for each weight group are also given. We shall have occasion to refer to this value later.

It will be noted that in this series of guinea-pigs, for weight groups 300-340 gm., and 340-360 gm., almost the same numbers of guinea-pigs die in less than the mean death period that die in a longer period. The average deviations from the mean, above and below, are practically equal. The mean death periods themselves for the two groups are almost the same, both being a little less than the ninety-six hours defined as the official death period in testing—a fact which would lead to the slight underestimation of the unitage of sera tested by this method.

This equal distribution of the death periods of guinea-pigs on each side of the mean led me to inquire into the relation of group varia-

TABLE 6
AN ANALYSIS OF TABLE 2 OF BULLETIN 43 SHOWING VARIATIONS OF DEATH PERIOD OF PIGS ON THE UNITAGE DETERMINING DOSE

Weight Group in Grams	Number of Guinea-pigs in Group	Average Weight in Grams	Mean Death Period in Days Hours	Average Deviation of Death Period				Greatest Deviation of Death Period				Time Between Extremes of Variations in Days Hours
				From Mean in Hours		From Standard (96 hr.) in Hours		From Mean in Hours		From Standard in Hours		
				Over	Less	Over	Less	Over	Less	Over	Less	
280-300	6	289	4 00	12 (2 Guinea-pigs)	9 (3 Guinea-pigs)	12 (2 Guinea-pigs)	9 (3 Guinea-pigs)	14 (3 Guinea-pigs)	13 (3 Guinea-pigs)	14 (3 Guinea-pigs)	13 (3 Guinea-pigs)	1 3
300-340	18	315	3 18	16 (7 Guinea-pigs)	10 (10 Guinea-pigs)	16 (5 Guinea-pigs)	14 (13 Guinea-pigs)	37 (5 Guinea-pigs)	22 (7 Guinea-pigs)	31 (7 Guinea-pigs)	28 (7 Guinea-pigs)	2 11
340-360	14	350	3 21	11 (7 Guinea-pigs)	10 (7 Guinea-pigs)	11 (5 Guinea-pigs)	13 (7 Guinea-pigs)	22 (1 Guinea-pig)	19 (1 Guinea-pig)	19 (1 Guinea-pig)	32 (1 Guinea-pig)	1 17
360-280	4	374	4 6	61 (1 Guinea-pig)	20 (3 Guinea-pigs)	67 (1 Guinea-pig)	14 (3 Guinea-pigs)	61 (1 Guinea-pig)	32 (1 Guinea-pig)	67 (1 Guinea-pig)	26 (1 Guinea-pig)	3 21

tion to individual variation as illustrated by this series. Table 7 shows the results of this inquiry. Herein it is shown that in 79 percent of the sets of guinea-pigs of the same weight group on the same testing (eleven sets out of fourteen), all the guinea-pigs in the set died in less time than the mean death period for the weight group, or else all died in a longer period. That is, the variation seems to be a group reaction rather than a variation of pigs within groups, altho remarkable instances of this latter occur and must be expected, as shown in Table 8.

TABLE 7
AN ANALYSIS OF TABLE 2 OF BULLETIN 43 SHOWING GROUP VARIATION

Number of Guinea-pigs in Set	Number of Guinea-pigs Dying in Mean Death Period or More	Number of Guinea-pigs Dying in Less Than Mean Death Period	Weight Groups in Which All Guinea-pigs in Each Set Belong
1.....	1	0
2.....	2	Same
3.....	3	Same
4.....	4	Same
4.....	4	Same
2.....	2	Same
2.....	2	Same
2.....	2	Same
2.....	2	Same
2.....	1	1	Same
2.....	2	Different
2.....	2	Same
2.....	1	1	Same
2.....	2	Same
2.....	1	1	Different
2.....	2	Different
2.....	2	Same
1.....	1
3.....	2	1	Same

TABLE 8
VARIATIONS WITHIN GROUPS AS SHOWN IN TABLE 2 OF BULLETIN 43

Weight-Group	Extreme Variation of Death Periods Within Sets, All Guinea-pigs in Set Being Tested at the Same Time
280-300	7 hours
300-340	22 hours
340-360	23 hours
360-380	12 hours

A FURTHER CONSIDERATION OF GROUP VARIATION

So long as the factors involved in the standardization of biological products are as variable as they now are, it would seem to be advisable to take every step possible to aid in reducing these variations to a minimum. In order to avoid the anomalies which are so often

encountered among individual guinea-pigs, in every set of guinea-pigs in the testing of a serum, there should be at least four individuals from the same source, preferably from the same litter, on different doses. It would probably be an unnecessary refinement to put a series of guinea-pigs on the same dose, if the proper precautions are taken. Amongst these precautions are, that all pigs should be kept under the same conditions of housing and feeding before the test, and that especial care should be observed in handling or disturbing the animals which show symptoms that are severe. Even a gentle handling, or the sudden banging of a door, may result in the premature death of a tetanized animal which otherwise would have lived for perhaps half a day or more longer.

TABLE 9
POSSIBLE GROUP VARIATIONS IN TESTING ANTITETANIC SERA

Volume of Serum	Time of Death or Symptoms		Theoretical Unitage	Indicated Unitage	Extreme Variation
	Test I	Test II			
$\frac{1}{11,100}$	70 hr.	32 hr.			
$\frac{1}{9,400}$	96 hr.	60 hr.	940 units (Test I)	
$\frac{1}{8,000}$	115 hr.	74 hr.	800 units	32½ percent
$\frac{1}{6,800}$	140 hr.	96 hr.	680 units (Test II)	
$\frac{1}{5,780}$	168 hr.	144 hr.			

But even if individual variation were reduced to a minimum, we must still consider the variation of the group as a whole. As MacConkey³ has shown, working with English-bred guinea-pigs, group variation, in many cases, makes a considerable difference in the indicated unitage of a serum. This fact is well exemplified in Table 9, reconstructed from the preceding tabulations, in which is shown what might happen in an extreme (but possible) case in which tests of a single serum were made at two different times. The fundamental variation in death period, (74-115 hours for the 1/8,000 c.c. of serum, i. e., an extreme variation between individuals of different sets on the

3. Jour. Hyg., 1913, 13, p. 467.

same dose of one day seventeen hours) is taken directly from Bulletin 43. The others are derived from Table 2.

This is a source of variation which should be eliminated by paralleling each test of a serum of unknown unitage with an exactly similar test on a standard serum. This is the general practice in the testing of other antisera in which group variations are anticipated. MacConkey³ has already suggested the extension of the practice to include antitetanic sera, a recommendation which finds ample support in the present paper.

SUMMARY

In a series of pairs of guinea-pigs weighing 300-340 gm., or 340-360 gm., injected at the same time with toxin-antitoxin mixtures containing the L + dose of toxin plus amounts of the same antitoxin having a definite ratio to each other, there are broad relations between the times of death or the symptoms of the two guinea-pigs, as expressed by this ratio between the two quantities of antitoxin.

There are many striking variations from these central types.

Resistance to unbound tetanus toxin offered by members of different weight-groups varies, but does not vary constantly according to weight.

There is an approximate parallelism in their resistance to unbound toxin, between the weight-groups 300-340 gm., and 340-360 gm. Their average curve, showing the average resistance of guinea-pigs weighing 300-360 gm. to graduated doses, is quite smooth.

The reaction of a group of guinea-pigs to the injection of a toxin-antitoxin mixture containing several times the M. L. D. of unbound toxin, is more constant than the reaction of a similar group of animals to the injection of a similar mixture containing just one fatal dose, or a little more, of unbound toxin. This supports the choice of the ninety-six hour death period.

From the death period of a test animal dying in less than ninety-six hours, it is possible to compute with fair accuracy what the unitage-indicating dose should be in the next testing of the same serum.

In testing antitetanic sera by the American method, in which parallel tests on a standard serum are not made, testing the unitage of a serum of unknown potency any closer than 15 percent should not be attempted.

Group-variation of the resistance of test animals is of great importance. The difference in indicated unitage of the same serum can, in extreme cases, be as great as 32 percent, and even under the most favorable conditions, as shown by the results given in Bulletin 43 of the Hygienic Laboratory, is to be reckoned with.

A standard tetanus antitoxin should be distributed for use in parallel tests in all standardization of antitetanic sera by the American method.

STUDIES ON THE GONOCOCCUS. III *

CARL C. WARDEN

(From the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.)

AUTOLYSIS

In previous papers I have called attention to lysis in the gonococcus and have emphasized this phenomenon as being invariably present to a greater or less degree in artificial culture. This study was undertaken with the objects of ascertaining which factors bring about and influence the lytic changes—whether the process is due wholly to the action of enzymes, as is commonly maintained, or to other causes—and of obtaining a more intimate knowledge of the biochemistry of the organisms. This has seemed to me important in view of the practical value such observations might have in the problem of immunity. The material consisted of twenty-two strains of gonococcus isolated by me within the past year from cases of gonorrhea in men and young girls, in addition to several, including six strains of meningococcus, kindly given me by other workers.

The question first to be considered was whether lysis might be due to conditions in the culture media, or to causes within the organisms themselves. Accordingly the strains were grown upon all media specially recommended for cultivating the gonococcus, with the result that all strains showed lysis upon all media,¹ varying only in degree under conditions to be mentioned later. This fact being determined there remained to be shown what element of the media, common to all, initiated the lysis, on the assumption that the conditions of environment were alone responsible.

In order to avoid a multiplicity of media a standard was adopted consisting of salt-free veal broth containing 2 percent agar, neutralized to phenolphthalein, to which was added 5 percent defibrinated rabbit blood.

The optimum reaction was observed by varying from the neutral point. The results showed that while the organisms could be brought to endure considerable variation in reaction, the best condition for all strains was at the neutral point, altho at all points lysis occurred.

The quantity and quality of nitrogen were next investigated. The quantity of nitrogen in the medium was reduced first by eliminating the blood, the

* Received for publication March 19, 1915.

1. Jour. Infect. Dis., 1913, 13, p. 124.

strains being grown upon salt-free, neutral veal peptone agar. This medium gave satisfaction for the propagation of the strains but permitted lysis to occur. The peptone was then omitted from the medium with the same results. This medium has been used for some time by Park and Williams. I have found it serviceable for all strains after the primary culture. While the nitrogen content of this medium is probably complex, it is extremely simple in comparison with the elaborate supply and character in the standard. From this point in the study all transplants were made from this medium. The time limit for observations on lysis was twenty-four hours. Cultures older than one day contain many dead organisms.

The veal was next replaced by nitrogen-bearing salts covering a wide range. Such substances as asparagin and simpler salts, such as potassium nitrate and sodium nitrite, were used successfully in propagation, all cultures being carried through eleven transplantations at intervals of forty-eight to seventy-two hours. It was found that upon such extremely simple media as 2.25 percent agar in distilled water, containing no other substances than small amounts of sodium hydrate or sodium carbonate, the organisms found sufficient nitrogen to maintain existence, but in every case, save as shown later, lysis was noted. Evidently, lysis is not influenced by the amount or character of the nitrogen in the medium.

Coincidentally with the nitrogen examination, the veal agar cultures and those of simpler nature were subjected to various degrees of diminished oxygen. Novy jars were used entirely and the oxygen lowered with pyrogallol-sodium hydrate. All cultures, even on the simple media mentioned, were gradually accommodated to full anaerobiosis (10 gm. pyrogallol and 50 c.c. of 25 percent, sodium hydrate solution per liter of space), and continued in this environment for the full eleven generations. Under these unusual conditions the cultures showed no variation in lysis or microscopical appearance from the standard cultures, whereas the gross appearance changed considerably. On the simplest media the growth became very delicate and vapor like, the colonies being visible only with a hand lens and never exceeding a diameter of 0.5 mm. From these cultures transplantation back on the standard medium was made whereupon they resumed the ordinary character of growth. It was also found that variations of temperature within limits of viability produced no change in lysis.

It will be observed that this range of investigation has excluded any possible influences of sugar and fat upon lysis and there remains therefore but one factor in the media for consideration, namely, water. Previous experiences with fluid media had shown me that in them lysis appeared to be greatest. In working with solid media also it was noticed that growth in the more moist regions of the media always showed a greater degree of lysis. This was apparent also in smear preparations for microscopical examination when the substance was taken from the moist portions of the plate or near the water of condensation in the tube. Slight variations in detail of making smear preparations create great changes in microscopical appearance. A simple experiment will illustrate. Remove a trace of material from the edge of a twenty-four-hour colony of gonococcus or meningococcus, smear it quickly upon a slide with a very minute drop of water or salt solution, and dry immediately with an air blast or by blowing sharply on the slide. Upon the same slide make smears in one loop of water, then in two loops, then in three or more pooled, and allow to dry in the air. After fixing and staining with methylene blue or, better, dilute carbol-fuchsin, examination in series will show the extraordinary changes which excess of water will produce in a very short time. The alterations in morphology and

other changes indicative of lysis are too well known to require detailed mention. Briefly, the process begins with increase in size of the coccus and is followed by distortion into a great variety of forms, together with granulation, accompanied from the beginning by diminished brilliancy of staining, extending from blurring to the faintest shadows.

The gonococci may derive excess of water while on a medium from sources other than the medium, as for instance from a very moist atmosphere, from water of condensation, including that which occurs incident to the removal of glassware from incubator temperature into that of the room. The effect of the latter may be shown by placing plates bottom up or agar slants culture surface uppermost after removal from the warm room, when the vapor will form on the growth instead of on the glass. In a word excess of water causes lysis. It will be objected at once that the contention will not hold for the reason that gonococcus and meningococcus will develop in fluid media, and also by reason of the observations of other workers that this group of organisms is very sensitive to lack of moisture.² The first objection is admitted. Very many organisms may be induced to propagate upon media ordinarily harmful, and the gonococcus-meningococcus group is no exception. However, my observations have shown that nearly all pure strains avoid the fluid as far as possible by building their growths on the surface and that all cultures in fluid media show extreme lysis, large, poorly staining cocci carrying their maximum load of water, and that transplants from the body of the fluid, which is usually clear when strains are uncontaminated, or from the bottom of the culture tube, are successful only very rarely, probably when fresh particles from the surface growth are settling or have recently reached the bottom.² (In defibrinated human blood the cocci may live for a long time). It is admitted also that these cocci adapt their existence to considerable degrees of moisture on solid media with the result that often more luxuriant growth is so obtained, as the existence depended on rapidity of vegetation. I believe, however, that these exceptions merely prove the rule, for my experience has been that such cultures always show great lysis and are very prone to perish overnight. When in this "water logged" state, the cocci are very sensitive to injury so that rough handling often kills a transplant.

With a view to determining whether an optimum of water would permit growths of this group free from lysis on the medium, a long series of observations was made with many media in varying degrees of dryness. To avoid details the results may be summarized by stating that all strains were cultivated with greater surety and uniformity and with least lysis upon media containing a minimum of moisture. Success with the simple media mentioned, with and without oxygen, depended on attention to this detail. I now use but two media for routine cultures and for isolation, one the salt-free neutral veal 2.25 percent agar, and the other the same to which while at 60 C. is added 2.25 percent defibrinated rabbit blood. Primary cultures are made in the latter and are transferred at once to the former. Before use the

2. Irons: *Forscheimer—Billings, Therapeutics of Internal Diseases*, 1914, 5, p. 599. Ohlmacher: *Jour. Am. Med. Assn.*, 1915, 64, p. 585.

tubes are dried in desiccators over sulphuric acid or in a dry room at 37 C. until all water of condensation has gone and a point is reached where examination of the surface of the medium with a hand lens shows a slight roughening or puckering. If the agar has cracked, no harm is done. From such dry media the organisms get all the water they require and upon these media a twenty-four-hour growth shows vegetation without lysis. I have found that by the use of the dry blood medium the chief difficulty in securing primary cultures has been overcome. A minimum amount of pus gently distributed over the entire area insures colony formation, whereas on more moist media very often the culture fails. As regards the maintenance of growth, transplants may be made once a week, altho cultures frequently survive for many months, enduring until the medium has dried down nearly to the glass. In such growths secondary colonies or masses appear on the surface of the early growth, spread irregularly, and form cones which slowly flatten out and afford a base for other cones. In this way elevations of 3-5 mm. are built up, from the summits of which successful subcultures may be made. In transplanting, some idea of the requisite dryness of the medium may be had by observing that the material adheres to the surface wherever it comes in contact. If the surface is too moist the material, like fat, slips over it and rolls up into ridges.

Lysis in suspension in water, salt solution, or various other solutions, depends for the most part on two factors: the concentration of the suspension and the ionic content of the solution. In salt solution, lysis occurs in all concentrations, becoming progressively less up to 25 percent, beyond which a slower disintegration occurs. Sodium fluorid checks lysis in about 10 percent solution. Flocculation is readily produced by salt solutions at 0.9 percent and above, by solutions of the heavy metals, by acid ions, and in nearly all dilutions by the salts of the alkaline earths. Compact flocculation as with CaCl_2 delays lysis, while slight flocculation as with normal salt solution tends rather to increase it. The hydrosol colloids permit lysis apparently in direct proportion to the available water. Suspension colloids such as serum and lecithin-water favor lysis.

Those agents which prevent lysis have been well reviewed in recent literature. Many dehydrating substances such as 50-95 percent alcohol (McClintock and Clark), 25-50 percent acetone, strong solutions of

sugar (syrup), preserve the cocci for considerable periods of time. These substances extract certain ingredients from the organisms, especially alcohol, the effect of which will be mentioned later. While weak solutions of the alkali oleates do not prevent lysis, stronger solutions prevent it. Fatty substances, such as neutral fat, olive oil, lanolin, certain cosmetics, hydrocarbons like vaselin, and all substances more or less free from water, prevent lysis. From the surface of lanolin whereon small amounts of culture material had been placed, I have, after a month, obtained microscopical preparations showing the cocci to be in as perfect condition as to shape, size, and staining reactions as if taken fresh from culture. A point in this connection of greater importance is the fact that the viability of gonococci may be maintained in such substances for days. Small loops of gonococcus culture were planted upon lanolin sterilized and slanted in tubes. After five days at room temperature successful cultures were obtained on the standard medium; also from surface and buried inoculations on "cold cream," cultures were recovered on the fifth day. Plants on vaselin (Kahlbaum's white) at 20 C. and at 4 C. have yielded cultures after forty-eight hours. The behavior of culture material on such substances depended on the degree of moisture present in the culture. Wet material, after planting, gradually ran together into small, colony-like masses, while dry, somewhat granular material remained as placed. Such masses are easily distinguished from droplets of condensation. In culturing from such substances there were many failures because of the difficulty in freeing the organisms from the fatty envelope.

Glycerol 75-100 percent prevents lysis and preserves the cocci best of all substances examined. The appearances remain unchanged after months in this menstruum. Lysis occurs in 50 percent glycerol. The behavior of gonococcus and meningococcus, especially the former, in suspension in glycerol is peculiar. Small loops of culture material were suspended in as fine a state of division as possible at the surface of sterile pure glycerol in tubes kept at 4 C. and at 20 C. After two or three days the particles near the surface were evenly distributed in fine suspension, below which was a layer of coarser granules out of which floccules of larger size had separated and were settling, leaving delicate, white streamers in their track. These white, granular looking floccules appeared to increase in size as they settled in the

fluid. Microscopic examination showed them to consist of masses of cocci of perfect shape and stain-power, apparently undergoing growth. Individual floccules, pipetted into fresh glycerol, repeated the process on a smaller scale. Floccules from gonococcus transferred to dry blood agar from glycerol tubes kept at 4 C. for seventy-two hours, yielded abundant colonies, and scattered colonies were obtained from tubes kept at 20 C. for six days. The cocci are extremely sensitive to injury in glycerol, as may be seen in microscopical preparations that are made at all roughly. The addition of 1 percent boric acid to glycerol made no difference in viability of the cocci. Cultures of the meningococcus were not made. A small drop of glycerol containing a gonococcus floccule from a tube kept at 20 C. for forty-eight hours was spread over a small area on the abductor surface of the last phalanx of the little finger. From this an impression was made after one hour on dry blood agar. Incubation for forty-eight hours yielded a single gonococcus colony from which sub-cultures were made. Successful cultures were never obtained from glycerol kept at 37 C. after twenty-four hours. The dry blood agar absorbs considerable amounts of glycerol in a short time. All these observations lead to the conclusion that lysis of this group of organisms is initiated by water in excess.

The next inquiry concerned the nature of the changes in the gonococcus induced by water, and to this end it was necessary to examine the normal organic substances in the organisms. My attention was drawn first to the fats by several observations, namely: the penetrating and characteristic odor of some samples of freshly dried gonococcus substance, the relation existing between the gram character of many bacteria and their lipid content,³ the fact that gonococcus suspensions and autolysates showed on shaking a more or less permanent foam due partly to proteid and partly to soaps, and the presence in salt solution autolysates and suspensions of a lipase which produced acid from neutral fat (olein).

QUALITATIVE EXAMINATION OF THE FATS

Large 24-36 hour cultures of gonococcus grown on fat-free medium (slightly acid veal broth extracted with ether, neutralized to phenolphthalein 2.25 percent agar) were saponified and the solution after acidifying was distilled in steam. The early fractions of the distillate showed fine, oily drops on a clear fluid, later fractions gave a slightly cloudy distillate carrying abundant large, white, floating flakes, considerable quantities of which accumulated in the condenser, while the last portions to distill over were clear.

3. Tamura: Ztschr. f. Physiol. Chem., 1914, 89, p. 304.

The entire distillate, after the addition of a slight excess of alkali, was evaporated to dryness on a bath and the residue taken up in a small quantity of 20 percent sulphuric acid. Certain higher fatty acids appeared on the surface as oily drops and solid flakes. By siphonage the acid solution was separated and, on salting, showed still lower volatile fatty acids, and extraction of the salted solution with ether yielded appreciable traces of others.

The non-volatile acids remaining after distillation were then recovered by extraction with ether. At 20 C. these were solid, yellowish substances with the same pungent odor as that of the dry substance.

By the lead-salt-ether method the unsaturated fluid fatty acids were separated. These were dark red in color, with slight odor. The remaining saturated fatty acids were solid, rather bulky, and white.

I have been unable to identify these acids. Certain of the volatile acids solidify from ethereal solution at 12 to 13 C. and melt at 6 to 5 C. The melting point of the non-volatile saturated acids could not be determined exactly, 43 to 45 C. being the figures oftenest obtained.

To summarize, the gonococcus yields considerable quantities of rather bulky fatty acids, volatile and soluble in water and salt solution, non-volatile, saturated and unsaturated.

QUANTITATIVE ESTIMATION OF THE FATS

These estimations have yielded amounts which varied considerably with the physical state of the culture material and the method of treatment. Washing and drying produce great changes, as do also the age of the culture and the amount of moisture it contains. Accordingly in each estimation the state of the substance is noted.

Twenty-four-hour mass cultures of gonococcus grown on fat-free medium were washed repeatedly with water, then dried in vacuo over P_2O_5 and powdered. By saponification method, the following results were obtained:

Weight of Substance	Volatile Acids	Unsaturated Acids	Saturated Acid
2.0140	0.0279	0.0067	0.0549

This analysis was selected from many as representing an average of fatty acids yielded by substance prepared in this way. As will appear later, these figures are too low, especially for the volatile and unsaturated fatty acids. In using smaller quantities of substance desiccation by alcohol was tried, but abandoned when it was found that alcohol removed rather large amounts of fat.

In order to determine approximately in what condition the fats exist in the unaltered gonococcus, a thirty-six-hour culture was washed once in water, centrifugated at 6,000 and the sediment shaken out with petroleum ether, 50 percent alcohol, the solutions separated, the ether solution shaken with fresh 50 percent alcohol, and vice versa, several times, and the appropriate solutions added together. At the same time a washed and dried specimen was examined by the same method. The results follow:

Weight of Substance	Neutral Fats	Fatty Acids and Soaps
Wet	0.0133	0.005
Dry	0.054	0.003

The water in which the fresh culture had been washed yielded fatty acids 0.0076.

A control estimation shows that not all the fats are obtained by such a method. Air-dried powdered gonococcus substance was extracted with petroleum ether in the cold by long grinding and shaking. After separation the ether was treated as above. The residue was then extracted with fresh petroleum ether, on a water bath, with a condenser, for two hours and the ether again separated. The residue was finally saponified. The results were:

Weight of Substance	First Filtrate	Second Filtrate	Residue
2.8464	Fats 0.0636 Acids 0.0047	0.0196 0.008

To determine the amount of fat liberated by the gonococcus on suspension in water, the following experiment was made. The growth of thirty-six-hour cultures was received into distilled water and divided into equal portions. One portion stood at 20 C. for two hours and the other was heated at 65 C. for the same length of time. Both portions were then centrifugalized at high speed until sedimentation was complete. Fluids and sediments were then examined. As control, a quantity of culture equal to each portion was quickly removed into alcoholic potash, saponified and estimated, with results as follows:

Temperature	Volatile Fatty Acids	Non-Volatile Fatty Acids
20 C. Fluid	0.0072	0.0018
Sediment	0.0244	0.0114
65 C. Fluid	0.0216	0.0036
Sediment	0.0156	0.0078
Controls 1.	0.0238	0.0174
2.	0.0299	0.0403

The amount of non-volatile acids obtained for Control 2 was obtained by raising the boiling point of the acidified solution and adding a trace of CuSO_4 as catalyst. This shows that a considerable quantity of fat is not readily dissociated by ordinary means and that the figures before given for the non-volatile fatty acids are too low. Further work has led me to believe that at least a part of the increased yield is due to unsaturated fatty acids in a lipid combination which is not hydrolysed until the nitrogen is thoroughly broken up.

The following table indicates that the fats of the gonococcus probably differ from the fats of other organisms in character and proportion. Cultures from equal surface areas were used:

Organism	Volatile Acids	Unsaturated Acids	Saturated Acids
Staphylococcus	None	0.0032	0.0016
Anthrax	None	0.0146	0.0086
Typhoid	0.008	0.0049	0.0144

To obtain an idea of the proportion of fatty substances of the gonococcus extractable by ether and alcohol and the relation of these substances to the nitrogen, the following analyses were made. In testing small quantities of material wherein the nitrogen content is expressed in milligrams, adaptations of the micro methods of Folin were found serviceable.

A quantity of air-dried gonococcus substance, finely powdered, was extracted with a large volume of ether in the cold, with frequent shaking for twenty-four hours. The clear, colorless extract yielded on evaporation a white, fat-like body. Re-dissolved in ether, the solution gave a faint cloud with platinic chlorid but none with acetone. The gonococcus residue, after ether extraction, was then extracted with 99 percent alcohol at room temperature, with frequent changes, for seventy-two hours. The extract was clear, slightly yellow, and deposited a reddish residue soluble in absolute alcohol. The solution gave a white cloud with water and with acetone, and an abundant yellow precipitate with platinic chlorid.

The gonococcus residue after alcohol extraction was then saponified and the remaining fats recovered as fatty acids.

CONTROLS

- 0.2002 air-dried gonococcus gave 0.0256 total nitrogen (Folin).
0.35 air-dried gonococcus gave 0.0415 total nitrogen (Kjeldahl).
0.501 air-dried gonococcus gave 0.0647 total nitrogen (Folin).
0.501 air-dried gonococcus gave 0.0261 fatty acids.

The calculated total nitrogen in 3.0105 gonococcal substance was 0.3853, the calculated fatty acids 0.15. Quantity of alcoholic extract, 130 c.c.; total nitrogen in 10 c.c. of extract, 0.000197; calculated total nitrogen in extract, 0.002575. Total fatty acids in 10 c.c. extract, 0.0023; calculated fatty acids in extract, 0.03. Ether extract gave 0.0001 total nitrogen and 0.0184 fatty acids. Residue by saponification gave fatty acids, 0.0279.

There is a discrepancy in the fats which is not explained.

A second record is submitted of a similar analysis save that ether extraction was omitted.

Calculated total nitrogen in 3.1975 gonococcal substance, 0.4093; fatty acids 0.166. Quantity of alcoholic extract, 130 c.c.; determined nitrogen in 10 c.c. extract, 0.0002; calculated nitrogen in 10 c.c. extract, 0.002624. Determined fatty acids, 0.005; calculated fatty acids, 0.065. Residue by saponification, 0.068.

From these analyses it is observed that extraction of dried gonococcus substance with ether in this manner yields a small proportion of fatty substance containing a trace of nitrogen, while alcoholic extracts yield about one-half the total fats containing less than 1 percent of the total nitrogen. The inference is that some of the nitrogen is closely bound to the fat in the gonococcus.

Having observed the extent to which lysis of gonococcus in water suspension liberated fats, I deemed it advisable to ascertain to what extent and in what form nitrogen is set free. If lysis were due to enzyme activity, the nitrogen would probably be, at least in part, present as ammonia. Accordingly many tests were made but it was found that little information could be gained by estimating NH_3 . All suspensions and autolysates showed NH_3 in measurable amounts which apparently increased with the time, at moderate temperatures, and diminished at high temperatures. Of the many suspensions examined those heated to 58 C. for thirty minutes, then kept at 37 C. for forty-eight to seventy-two hours showed the largest quantities of nitrogen as NH_3 in the autolysates.

LYSIS IN RELATION TO TOXICITY AND ANAPHYLAXIS

In order to observe the degrees of lysis in sera and the property of suspensions of gonococcus undergoing lysis for the production of anaphylatoxin in sera, the following experiment was made.

The cultures used were dry, twenty-four-hour agar-grown, and to avoid previous lysis were emulsified directly in the sera. The serum suspensions contained no agar. After incubation at 37 C. the suspensions were centrifugalized until perfectly clear of cocci (4,000 revolutions for 20 to 30 minutes) and injected intravenously into guinea-pigs, weighing 225 to 250 gm. each.

TABLE 1.
THE EFFECTS OF INJECTION OF GONOCOCCI IN VARIOUS SERA

Guinea-Pig	Quantity of Culture	Serum	Hours at 37 C.	Amount Injected	Results
1	0.5 slant	Normal rabbit	1.5	4 c.c.	Mild shock, ill later, died 12 hours Autopsy negative
2	0.5 slant	Inactivated rabbit	1.5	4 c.c.	No shock, ill later, lived
3	0.5 slant	Immune rabbit (old)	1.5	4 c.c.	Itching, ill later, lived
4	0.25 slant	Immune rabbit, reactivated	1.5	4 c.c.	Severe shock, ill later, died 10 hours
5	0.25 slant	Normal guinea-pig	1.5	4 c.c.	Slight shock, ill later, died 8 hours
6	0.25 slant	Immune goat (old), react.	1.5	4 c.c.	Slight shock, ill later, lived
7	0.25 slant	Normal guinea-pig	0.5	4 c.c.	No shock, ill later, lived
8	1 slant	Normal guinea-pig	3	4 c.c.	Severe shock, ill later, died 8 hours
9	1 slant	Normal guinea-pig	4	4 c.c.	Death 2 minutes; typical anaphylaxis with emphysema, blood fluid
10	1 slant	Normal guinea-pig	4	4 c.c.	Death 2.05 minutes; anaphylaxis
Control	Normal rabbit	4	4 c.c.	No effect
Control	Normal guinea-pig	4	4 c.c.	No effect

The next experiment was made to determine whether the late death of the animals was due to poisonous substances from the gonococcus alone. The suspensions in water and salt solution were, as in the preceding, uncontaminated and were treated in the same manner as the serum suspensions. The injections of 4 c.c. were given intravenously into guinea-pigs of the same average weight. Salt solution was used by preference to facilitate centrifugalization.

From this experiment it was concluded that the late death of the animals was probably due to substances derived from lysis of the gonococcus.

Table 3 shows approximately the nitrogen values of such suspensions and autolysates as were used in the preceding experiment, together with others subjected to wider ranges of temperature. The same quantities of material were employed and the emulsions prepared in the same manner. The results are expressed in fractions of a milligram.

The results embodied in the tables, together with many control and duplicate experiments for which records are not given, show several points of interest.

It is possible to induce anaphylaxis easily in guinea-pigs with gonococcus suspended in homologous serum. Rabbit serum does not answer so well because of the large quantities of material necessary, and human serum is too toxic for guinea-pigs to be serviceable.

TABLE 2
THE EFFECTS OF INJECTION OF GONOCOCCI IN WATER AND SALT SOLUTION

Guinea-Pig	Culture	Fluid	Temperature	Time	Results
11	1 slant	Distilled water then salt to 0.9	37 C.	4 hours	Paralysis, temporary; quick recovery, ill, died 24 hours
12	1 slant	Salt solution	37 C.	0.5 hours	Paralysis, temporary; quick recovery, ill, died 10 hours
13	1 slant	Salt solution	37 C.	0.5 hours	Paralysis, temporary; quick recovery, ill, died 8 hours
14	1 slant	Salt solution	37 C.	4 hours	Paralysis, temporary; quick recovery, ill, died 10 hours
15	1 slant	Salt solution	37 C.	6 hours	Paralysis, temporary; quick recovery, ill, died 18 hours
16	1 slant	Salt solution	37 C.	48 hours	No effect, ill later, lived
17	1 slant	Salt solution	58 C.	1 hour	No effect, ill later, lived†
18	1 slant	Salt solution	58 C.	0.25 hour	Paralysis, temporary; ill later, died 18 hours
19	1 slant	Salt solution	58 C.*	Paralysis, temporary; ill later, died 10 hours
20	1 slant	Salt solution	70 C.	1 hour	No effect, ill later, lived
21	1 slant	Salt solution	70 C. then 48 hr.	1 hour 37	No effect, slight illness later, lived
Control	Salt solution	20	No effect
Control	1 slant	Salt solution	20	Paralysis, ill later, died 6 hours

* Centrifugated at once.

† The salt solutions were heated to the temperatures indicated before the addition of the culture.

Fresh autolysates of gonococcus in water, salt solution, and serum are toxic for guinea-pigs and all other animals. Autolysates formed at moderate temperatures within short periods of time are fatal for guinea-pigs. The illness is identical with that observed following intraperitoneal injections of gonococcus suspensions,⁴ the invariable feature of which is a rapid fall in temperature.

The toxicity of autolysates apparently does not depend upon the quantity of nitrogen present but probably upon the character of the nitrogen in the early stage and is due to the anaphylatoxin producing power of substances derived from the cocci.⁵

4. Warden: Jour. Infect. Dis., 1913, 12, p. 104.

5. Dold: Bakterien Anaphylatoxin, Jena, 1912.

Lysis occurs in water and in salt solution at all temperatures below the coagulating point of proteid. At low temperatures, lysis is not at once manifest in the suspension but becomes apparent with centrifugation. As more of the proteid is coagulated, or otherwise altered by increasing temperature, the percentage of total nitrogen in the fluid diminishes. The rate and degree of lysis increase with the length of

TABLE 3
THE NITROGEN VALUES OF GONOCOCCAL SUSPENSIONS AND AUTOLYSATES

Culture	Temperature, C.	Time	Nitrogen		
			Amount of Fluid in Milligrams	Amount of Sediment in Milligrams	Amount of Suspension in Milligrams
24-hour dry	4	2 hr.	0.4	0.965
24-hour dry	4	2 hr.	0.55
24-hour dry	20	at once	1.20
24-hour dry	20	20 min.	0.726
48-hour dry	20	20 min.	Trace	0.8
48-hour wet* ...	20	20 min.	1.0	0.4
24-hour dry	20	20 min.	0.36	0.27
24-hour dry	20	2-3-4 hr.	0.55
24-hour dry	20	6 hr.	0.765 amino †
24-hour dry	20	6 hr.	0.37	0.51	0.65
24-hour dry	37	20 min.
24-hour dry	37	20 min.	0.92	0.19	0.714
24-hour dry	37	20 min.	0.44	0.37
24-hour dry	37	1 hr.	1.2	Trace
48-hour wet	37	1 hr.	0.43	0.38
24-hour dry	37	4 hr.	0.37	0.36
24-hour dry	37	72 hr.	0.65	Trace
24-hour dry	58	1 hr.	0.66	Trace
24-hour dry	58	2 hr.	0.44	Trace
24-hour dry	58	4 hr.	0.42	0.30
24-hour dry	70	1 hr. then 20 C.	0.26	0.27
24-hour dry	70	2 hr.
24-hour dry	70	1 hr. then 30 C. 48 hr.	0.17	0.19
24-hour dry	100	1 min. cooled to 20 C.	0.2	0

* Very moist culture, grown on wet medium.

† Van Slyke method.

time of growth and water content of the culture, and vary with the lytic character of the strain.

Old cultures and very wet cultures apparently yield larger amounts of nitrogen to the water in a short time than fresh and dry cultures.

The amounts of nitrogen in fluid and sediment never equal the total nitrogen of the culture material estimated directly.

The total nitrogen of suspensions appears to diminish in amount the longer the suspensions have stood.

Lysis occurs at temperatures that check the activity of enzymes. The degrees of lysis in the individual cocci as observed by microscope in these experiments were so difficult to distinguish that no attempt at classification was made. Lysis was great in most instances and in serum invariably extreme. In general it appeared that extremes of temperature afforded better appearances of preservation.

PREPARATION OF GONOCOCCUS ANTIGEN

The examination of water autolysates as prepared by me for gonococcus complement fixation tests, showed them to contain proportional quantities of the substances already mentioned. The antigens are made in two ways: one by allowing suspensions to stand at 20 C. for two hours, centrifugalizing at high speed, and preserving the fluid sterile; the other by heating the suspension to 58 C. for two hours, centrifugalizing, and heating the fluid at 75 C. for thirty minutes. When freshly prepared, one-half made in the manner first mentioned contained 2.3 mg. total nitrogen, 0.0181 gm. fatty acids and 0.00017 gm. phosphorus as pyrophosphate, while one-half made in the second manner showed little or no variation save in the reduction of the fatty acids about one-half. Examinations of old antigens showed the total nitrogen apparently to have been much reduced in amount, while the fats were correspondingly less. Similar changes have been noted in vaccines.

TOXICITY OF FATS AND EXTRACTS

The following experiment was made to determine whether the alcoholic extract of gonococcus substance and an alcoholic solution of the fatty acids were toxic for guinea-pigs. The solutions used were (1) the alcoholic extract of dried gonococcus known to contain per 10 c.c., 0.005 gm. fatty acids and 0.2 mg. nitrogen, and (2) an alcoholic solution of gonococcus fatty acids known to contain per 1 c.c., 0.005 gm. Varying quantities of the solution were diluted with salt solution in such a manner that after evaporation of the alcohol at 56 C. the total quantity for injection was 4 c.c. The material after thorough shaking varied from a slight milkiness to dense opacity. Injections were intravenous into guinea-pigs weighing 225-250 gm. each.

It appears that the solutions given by this method were not toxic, but that as heavy suspensions they might cause death mechanically, or by inducing toxicity in the serum of the animal.

DISCUSSION

From what is known of the gonococcus it may be safely assumed that the limiting layer is extremely sensitive to changes in surface tension and very permeable by water. The almost naked cell body is easily bruised and altered by physical states which appear to have slight or no effect on many other bacteria. Certain electrolytes, such as NaCl, favor the permeability of the cocci, while others, such as HCl and CaCl_2 , are more or less antagonistic to it. In a previous paper I stated that HCl prevented lysis. This is true only for a time, as the acid ultimately produces an increase of permeability.⁶ The

TABLE 4
THE EFFECT OF INJECTION OF ALCOHOLIC EXTRACTS OF GONOCOCCI

Guinea-pig	Solution	Results
22	No. 1, 1 c.c.	No effect.
23	No. 1, 5 c.c.	Twitching, slight paralysis, quick recovery.
24	No. 1, 10 c.c.	Death 2 min. pulmonary emphysema, blood clotted.
25	No. 2, 1 c.c.	No effect.
26	No. 2, 5 c.c.	No effect, slightly ill later, quick recovery.
27	No. 2, 10 c.c.	Forced respiration, lies on side, recovery in one hour.

substance of the cocci is regarded to be soft and delicate, the plasm being held together by bulky fats, some of them possibly in a fluid state, a certain proportion of which exist as fatty acids soluble in water or as compounds easily hydrolyzed.

All these factors contribute to the rapid imbibition of water. With the edema of the cells, or following it, there occurs an outpouring into the fluid of fats, of compounds containing phosphorus, and of nitrogen as proteid or proteid split products, and as ammonia. The occurrence of volatile fatty acids and amino nitrogen in fresh autolysates suggests a possible source in a leucin-like substance, one that is associated with advanced proteolysis. This assumption would imply either that hydrolysis is extremely rapid or that considerable amounts of such substance exist preformed in the cocci or about them. The evidence points to the immediate liberation by excess of water of many products representing past enzyme activity.

6. Osterhout: Science, 1915, 41, p. 255.

The process of lysis, as considered in this paper, is complete within a few minutes and constitutes a primary stage in the disintegration of the organism. This may be hastened by shaking and by centrifugalization. The substances liberated into the fluid now undergo fairly rapid qualitative and quantitative changes, but certain proportions of nitrogen and fat persist in one or another form for long periods. At the conclusion of this stage the substance of the cocci contains residual proteid which is not liberated at once by fresh fluid but appears to be somewhat slowly converted within the remnants of the cells into simpler form. There is, then, no constant increase of nitrogen in the fluid.

The existence of enzymes in the live cocci is well known. One is proteolytic, acting best in a slightly alkaline medium and destroyed at 56 C.; a second is fermentative, splitting dextrose, while a third splits neutral fat and does not, so far as I have been able to determine, possess reversible action. The activity of the latter appears to cease at 55 C. It will be seen from the foregoing tables that lysis occurs at all temperatures, even that at which proteid is coagulated and at points where enzyme activity is destroyed or at least checked. The toxicity of autolysates for animals is not checked at 58 C., but the temperature apparently hastens the lysis and carries the substances beyond the toxic stage more quickly than temperatures of 37 C. or 20 C.

The influence of the character of lysis on immunity will be discussed in a later paper.

CONCLUSIONS

Lysis of the gonococcus in water and in salt solution is probably due, not to the activity of enzymes, but to other causes, among which water permeability and solution of fatty substances play important parts.

Lysis is probably initiated by excess of water.

The gonococcus is capable of retaining viability for considerable periods of time in more or less anhydrous substances such as glycerol, lanolin, vaselin, and cold cream.

NATURAL HEMOLYSINS IN HUMAN SERUM*

JOHN A. KOLMER AND ARTHUR J. CASSELMAN

(From the Laboratory of Experimental Pathology, University of Pennsylvania, Philadelphia)

In conducting complement fixation work with human serum, we have commonly used the sheep hemolytic system, altho we knew its imperfection, namely, the presence of variable amounts of natural anti-sheep hemolysin in human serum. It is important, on account of quantitative factors, to use a hemolytic system which is free from this source of error, that is, the presence of variable amounts of the same hemolysin in the human serum as that used in the hemolytic system.

We are aware that many other hemolytic systems have been advocated, such as antihuman, antiox, and antihen, but it was our object to determine more definitely which were the more advantageous from this standpoint. Because of the infrequent occurrence of isohemolysins, the antihuman hemolytic system is the ideal one, except that it is somewhat difficult to prepare in rabbits an antihuman amboceptor of sufficiently high titer to avoid the influence of hemagglutinins.

In the Bauer and Hecht-Weinberg modifications of the Wassermann reaction, the natural antisheep amboceptor is utilized and an immune amboceptor is not added. While in some sera a proper dose of hemolytic amboceptor is already present, this factor is variable. Of course, in the presence of a large excess of amboceptor in a serum, hemolysis may be carried too far in any series of tubes containing that serum, in cases of only slight fixation of complement. In other words, with larger amounts of hemolytic amboceptor, smaller amounts of complement are necessary for complete hemolysis and these quantitative factors are of importance in complement fixation reactions. While, however, the importance of natural hemolysins must not be overestimated in view of the common presence of natural antisheep hemolysin in human serum and the widespread use and general satisfaction of an antisheep hemolytic system in the Wassermann syphilis and other complement fixation tests, it may be important on occasions and for certain work to use a hemolytic system in which quantitative relations are not disturbed by the presence of natural hemolysins. This may

* Received for publication, March 23, 1915.

be accomplished by removing the natural hemolysin or by adopting a hemolytic system free from this error.

While the literature contains numerous references to the presence of natural antishoop hemolysin in human serum, we were unable to find any literature bearing upon the question of natural hemolysins for the erythrocytes of other vertebrates since the publication of the summary made by Sacks,¹ which is incomplete and indefinite and a compilation of data gathered from different sources.

With the necessity for definite and accurate information in our own work we have examined a large number of human sera for natural hemolysins for the erythrocytes of the sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit, and guinea-pig by a method of titration which was used in a similar study of natural hemolysins in rabbit serum by Kolmer and Williams.²

PREPARATION OF MATERIALS

Before testing, the sera were inactivated by heating to 55 C. for thirty minutes, as in preparation of sera for the Wassermann reaction. This inactivated serum was then taken and diluted with nine parts of salt solution (0.85 percent) and used in the following amounts: 0.05 c.c., 0.2 c.c., 0.4 c.c., 0.8 c.c., 1.0 c.c., and 2 c.c., corresponding respectively to 0.005, 0.02, 0.04, 0.08, 0.1, and 0.2 c.c. of the undiluted serum. Complement was furnished by fresh guinea-pig serum, using 1 c.c. of a 5 percent dilution in normal saline. The corpuscles were used in dose of 1 c.c. of a 2.5 percent suspension in all the tests, with the exception of the rat cells, which were in 2 percent suspension.

TECHNIC

In Tube 1 were placed 0.05 c.c. of the 1:10 inactivated human serum; in Tube 2, 0.2 c.c.; in Tube 3, 0.04 c.c.; in Tube 4, 0.8 c.c.; in Tube 5, 1 c.c.; and in Tube 6, 2 c.c. Then, 1 c.c. of the guinea-pig complement and 1 c.c. of the corpuscle suspension and enough normal salt solution were added to make the total volume 4 c.c. After shaking, the tubes were placed in the incubator at 37 C. for two hours, removed, and allowed to stand in the refrigerator over night, and the results read in the morning.

CONTROLS

Natural amboceptor was not tested for in complement, but was removed from the guinea-pig serum by mixing it with the same kind of corpuscles used in testing for natural hemolysin and allowing absorption to take place in the refrigerator. Each serum was tested in maximum dosage with the corpuscles to make sure that all native complement was rendered inactive. With several sets of reactions, specific immune hemolysins were used with the sera to make certain that none of the sera was anticomplementary. The corpuscle suspensions were always prepared of fresh blood and controlled as to undue fragility by suspending a dose in salt solution.

1. Kolle and Wassermann: *Handbuch der pathogenen Microorganism*, 1913, 2, p. 793.
2. *Jour. Infect. Dis.*, 1913, 13, p. 96.

Natural Antisheep Hemolysin.—Table 1 shows that inactivated human serum contains considerable antisheep hemolysin, only 7 percent of sera examined being free from it. This fact is generally recognized and is the reason for many of the modifications of the Wassermann reaction.

TABLE 1
RESULTS OF EXAMINATION OF 150 HUMAN SERA FOR ANTISHEEP HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	1	9	24	65
0.02	25	10	28	37
0.04	40	12	20	28
0.08	53	14	14	19
0.1	58	18	7	17
0.2	64	20	9	7

Natural Antidog Hemolysin.—Table 2 shows that natural antidog hemolytic amboceptors are fairly common in human serum, being present in some degree in 82 percent of the sera examined.

TABLE 2
RESULTS OF EXAMINATION OF 25 HUMAN SERA FOR ANTIDOG HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	12	88
0.1	0	24	36	40
0.2	16	36	30	18

Natural Antiox Hemolysin.—The amount of antiox hemolytic amboceptor was much less than that of the antidog and antisheep amboceptor and on account of the comparative ease of obtaining ox blood in abattoirs and preparing highly potent immune antiox hemolysins, an ox hemolytic system is used in several laboratories and has been recommended for routine work.

TABLE 3
RESULTS OF EXAMINATION OF 85 SERA FOR ANTIOX HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	8	92
0.1	0	8	16	76
0.2	6	20	24	50

Natural Antigoat Hemolysin.—Of the twenty-five human sera examined for antigoat hemolysin, none showed any hemolysis in dosage less than 0.1 c.c., and none gave complete hemolysis with any of the doses used.

TABLE 4
RESULTS OF EXAMINATION OF 25 HUMAN SERA FOR ANTIGOAT HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	Percentage No Hemolysis
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	4	96
0.2	0	8	16	76

Natural Antihog Hemolysin.—Human sera rarely contain natural antihog amboceptor, only three sera exhibiting it, giving more than 25 percent hemolysis in only one case of 100 sera examined.

TABLE 5
RESULTS OF EXAMINATION OF 100 SERA FOR ANTIHOG HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	1	99
0.2	0	1	3	96

Natural Antirat Hemolysin.—When twenty-five sera were tested for hemolysis of the blood-cells of *Mus norvegicus*, in three cases 0.2 c.c. of serum gave 25 percent hemolysis, and so it may be deduced that human serum rarely contains this amboceptor.

TABLE 6
RESULTS OF EXAMINATION OF 25 SERA FOR HEMOLYSIS OF BLOOD-CELLS OF *MUS NORVEGICUS*

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	12	88

Natural Antichickens Hemolysin.—When twenty-five sera were tested for antichickens hemolytic amboceptors, only two cases exhibited any hemolysin, and then only 25 percent of the corpuscles were hemolyzed, even with 0.2 c.c. of serum.

TABLE 7
RESULTS OF EXAMINATION OF 25 SERA FOR ANTICHIKEN HEMOLYTIC AMBOCEPTOR

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	8	92

Natural Antihorse Hemolysin.—Of the twenty-five sera tested for antihorse amboceptor, 0.2 c.c. of serum gave 25 percent hemolysis with only one serum.

TABLE 8
RESULTS OF TESTS OF 25 SERA FOR ANTIHORSE AMBOCEPTOR

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	4	96

Natural Antirabbit Hemolysin.—The results of tests of twenty-five human sera against rabbit erythrocytes were the same as those for antihorse hemolysin.

TABLE 9
RESULTS OF TESTS OF 25 SERA AGAINST RABBIT ERYTHROCYTES

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	4	96

Natural Antiginea-Pig Hemolysin.—Table 10 shows the results of the examination of fifty sera for antiginea-pig hemolysin.

TABLE 10

RESULTS OF EXAMINATION OF 50 SERA FOR ANTIGUINEA-PIG HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	2	98

In this table we note that only one serum caused any hemolysis and, in that case, it was only partial: 25 percent with the 0.2 c.c. dose of serum. Owing to the fact that guinea-pig complement serum is used almost exclusively in hemolytic work it would appear that a guinea-pig hemolytic system should prove quite satisfactory because both complement serum and corpuscles are readily accessible. It is somewhat difficult, however, to produce a satisfactory rabbit immune hemolysin for guinea-pig cells, and this reduces the value of these erythrocytes in a hemolytic system.

Tables 1-10 give all the essential data, but it will be of greater interest to analyze the results more minutely. Tables 11 and 12 show the smallest amounts of human serum causing 100, 75, and 25 percent destruction of the red blood-cells of the various animals investigated and what percentage they bear to the number of sera tested.

TABLE 11

SHOWING THE SMALLEST AMOUNTS OF HUMAN SERUM GIVING 100, 75, AND 25 PERCENT HEMOLYSIS AND PERCENTAGE OF CASES IN WHICH IT MAY BE EXPECTED

Blood-Cells	100% Hemolysis	75% Hemolysis	25% Hemolysis
Sheep.....	0.005 (1%)	0.005 (10%)	0.005 (34%)
Dog.....	0.2 (16%)	0.1 (24%)	0.08 (12%)
Ox.....	0.2 (6%)	0.1 (8%)	0.08 (8%)
Goat.....	0.2 (8%)	0.1 (4%)
Hog.....	0.2 (1%)	0.1 (1%)
Rat.....	0.2 (12%)
Chicken.....	0.2 (8%)
Horse.....	0.2 (4%)
Rabbit.....	0.2 (4%)
Guinea-pig.....	0.2 (2%)

In Table 12 we have summarized the presence of natural hemolysins in 0.2 c.c. of human serum.

TABLE 12
SUMMARY OF NATURAL HEMOLYSINS IN HUMAN SERUM (0.2 C.C. AMOUNTS)

Blood-Cells	No. of Sera Tested	Percentage Showing 100% Hemolysis	Percentage Showing 75% Hemolysis	Percentage Showing 25% Hemolysis	Percentage Showing No Hemolysis
Sheep	125	64	20	9	7
Dog	25	16	36	30	18
Ox	85	6	20	24	50
Goat	25	0	8	16	76
Hog	100	0	1	3	96
Rat	25	0	0	12	88
Chicken	25	0	0	8	92
Horse	25	0	0	4	96
Rabbit	25	0	0	4	96
Guinea-pig	50	0	0	2	98

The obvious conclusion from Tables 11 and 12 is that the sheep and dog are not the ideal hemolytic systems for performing complement fixation work with human serum, but that hog, rat, chicken, horse, rabbit, or guinea-pig systems are preferable, depending from the practical side upon the ease with which we can obtain these corpuscles and their specific amboceptors.

AN EPIDEMIC, SIMULATING TYPHOID, CAUSED BY A PARAGAERTNER ORGANISM *

GEORGE H. ROBINSON

(From the Bacteriological Laboratory of Brown University and the Providence Health Department, Providence, R. I.)

Epidemics of typhoid fever due to the drinking of sewage-polluted water show a marked similarity in the ratio of those affected with gastro-enteritis to the cases of clinical typhoid fever. Comparison of several epidemics of this nature indicate that 40-60 percent of those exposed to infection contract gastro-enteritis and 2-8 percent develop clinical typhoid. In epidemics in which food or milk, infected by typhoid carriers or incipient cases of typhoid, are the vehicles of infection, the incidence of typhoid is much higher and the accompanying gastro-enteritis is generally absent. During the investigation of the following epidemic a study was made of the cases of enteritis as well as of those pronounced as typhoid.

In the fall of 1913 there occurred an epidemic simulating typhoid among the Rhode Island party attending the Perry Centennial at Put-In-Bay, Lake Erie. The itinerary of the party and the sanitary aspect of the epidemic are thoroughly discussed in De Valin's report¹ and that of Swarts.² The party, consisting of 422 members, left Providence September 8 and upon reaching Buffalo was quartered on two steamers, the Rochester and the Greyhound, 300 being accommodated on the former and 122 on the latter. The courses of both boats were practically the same and both parties attended the same functions. The delegation returned to Providence on September 14.

Reports from 235 members of the party on the Rochester showed 122 cases, or 52 percent, of gastro-enteritis. Of the total 300 on board 42, or 14 percent, developed clinical typhoid, including 6 deaths. The party on the Greyhound yielded upon inquiry 12 mild and temporary attacks of diarrhea" such as any change of water or diet might cause. In many ways this epidemic resembled others of infected-water origin, except that the number of typhoid cases was larger than the average.

* Received for publication March 26, 1915.

1. Publ. Health Rep., 1913, 28, p. 2761.

2. Health Bull. Rhode Island State Board of Health, 1914, 3, p. 1.

All possible sources of infection were carefully investigated by Dr. C. V. Chapin of the Providence Health Department, Dr. G. T. Swarts of the State Board of Health, and Dr. de Valin of the U. S. Public Health Service. The most salient points of the inquiry were: On September 11 the Rochester pumped water for drinking purposes from Lake Erie at points "considered decidedly unsafe;" during the trip a cook had performed his duties while suffering from typhoid fever; an electrician on the Rochester was taken sick with typhoid August 16; a maid and a water-tender both left the boat on September 20 feeling unwell and were later diagnosed as cases of typhoid. The maid died without giving a positive agglutination test with typhoid bacilli.

A check on the manner of infection is supplied in the case of members of the Newport Artillery, one of the organizations taking passage on the Rochester. The 83 members of this company were previously warned against the dangers of drinking water and so drank sparingly. None of them was affected with enteritis, but twenty-two of them developed typhoid symptoms about two weeks after their return. Eliminating those who drank little water, we find at least 122 cases of enteritis, or 80 percent, of the 152 members of the party who did drink freely of the water.

SYMPTOMS

The most common symptoms were enteritis, diarrhea, malaise, and fever. "Forty-three presented symptoms varying from a mild paratyphoid, to a perfect clinical, typhoid character." Four of the patients were admitted to the Rhode Island Hospital, extracts from whose records follow.

CASE 16.—Admitted Oct. 4. Diagnosis, typhoid fever with lobar pneumonia complication.

Patient began to complain of headache, abdominal distress, pain in back, and fever, September 25. Went to bed September 28 because he felt weak. Appetite poor, bowels regular. No nose bleed. No cough. Stated that he had had a similar attack six years before. Malaria four years before.

Well developed and well nourished. Face flushed, lips cracked. Tongue thickly coated on dorsum, red and moist at tips and edges. On chest and abdomen are a few circumscribed papules varying from the size of a pin to half the size of a pea, which disappear on pressure. No râles in chest. No murmurs of heart. Leukocytes 14,000, polymorphonuclears 81 percent. No agglutination of typhoid bacilli.

Oct. 6.—Irrational, tosses about in bed, gets up if not watched. Spleen not palpable.

Oct. 7.—More delirious, temperature 103 F.

Oct. 8.—Temperature 101 F. yesterday, during the night rose to 104 F.; pulse 130. Abdomen soft. No blood in stools. Impaired resonance in right lower lobe of lung. Died.

CASE 21.—Admitted Oct. 8. Diagnosis, typhoid fever.

Patient had headache and diarrhea with cramps, Sept. 14. This lasted four days after which he felt as well as ever except that he became tired easily. On Oct. 4, he went on a canoe trip and became thoroughly exhausted. Since then has had severe headache. No chills and no fever. Had typhoid fever with a relapse nine years ago.

No râles, no heart murmurs, no tenderness on abdomen, no glandular enlargement. Leukocytes 5,600, polymorphonuclears 63 percent, mononuclears 33.

Oct. 10.—Temperature reached highest point, 103.5 F.

Oct. 15.—Many typhoid bacilli in blood culture.

Oct. 20.—Temperature normal.

Nov. 4.—Discharged.

CASE F.—Admitted Oct. 4. Diagnosis, typhoid fever.

Felt sick on return trip Sept. 13. Headache, and later in the day watery and frequent bowel movements and vomiting. Had profuse diarrhea up to Oct. 4. Has cramp in abdomen before every bowel movement and pain in the middle of the back almost constantly. No blood in stools. Has lost about 8 pounds. No nose bleed. Bad taste, appetite poor. High fever.

No tenderness in abdomen. Spleen palpable. No rose spots. Leukocytes 6,400, polymorphonuclears 70.5 percent, mononuclears 28.

Oct. 7.—Only complaint is dizziness. Spleen greatly increased in size. Serum agglutinates typhoid bacilli.

Oct. 10.—General condition good. Fever declining.

Oct. 13.—Patient at times irrational and slightly delirious. Temperature again elevated. No sign of perforation or hemorrhage.

Oct. 16.—During last three days has been profoundly toxic. During last twenty-four hours heart has been failing. Died.

CASE 40.—Admitted Oct. 29. Diagnosis, typhoid fever.

Symptoms began Oct. 22 with headache and loss of appetite, slight cough, but no nose bleed; slight fever, no chills, no vomiting, no pain in abdomen or chest or joints or back, no night sweats; bowels moved frequently last four days. Headache and malaise. Took complete typhoid inoculation one year ago.

Abdomen has suggestive rose spots. No tenderness or rigidity or spasms. Lower end of spleen felt. Leukocytes 11,000. Serum agglutinates typhoid bacilli.

Nov. 2.—Blood culture negative. Comfortable. Diarrhea stopped.

Nov. 5.—Feeling well but temperature is again 103 F. at evening.

Nov. 8.—Is beginning to feel listless and a little weak; no headache and no abdominal pain.

Nov. 11.—Temperature up. Weak.

Nov. 21.—Temperature normal.

Dec. 11.—Uneventful recovery. Discharged.

AGGLUTINATION

The first agglutination of typhoid bacilli by the serum of any of the party was obtained September 28. This patient, however, had received prophylactic treatment for typhoid a few months previously, but during the investigation of his case it became evident that there was an infection of some kind among the members of the party. The last specimen of blood from this epidemic was sent in November 9. During this time 39 specimens of blood were received at the city and state health laboratories from those residing in or near Providence who attended the Perry Centennial.

These yielded the following results:

	City Laboratory	State Laboratory
Positive to <i>B. typhosus</i> alone.....	4	6
Partial to <i>B. typhosus</i> alone.....	2	0
Positive to <i>B. paratyphosus</i> (B) alone.....	0	1
Positive to both.....	2	4
Partial to <i>B. typhosus</i> and suspicious to <i>B. para-</i> <i>typhosus</i> (B)	0	1
Negative to both.....	8	11
Total	16	23

The large percentage of the cases which showed a reaction with *B. paratyphosus* (B) was noticeable throughout the epidemic.

EXAMINATIONS OF FECES

Specimens of feces were obtained from nineteen of the men in Providence. Through the efforts of Dr. C. V. Chapin specimens of feces and blood were obtained from the cook on the Rochester. About two months after the infection, through the kindness of Dr. Swarts, specimens were obtained from the convalescents in the Newport Hospital. A technic which had proven very successful was used in the examination of the feces. Small portions of the material were incubated in lactose peptone bile for six to twelve hours and then the bile culture was plated on Endo's medium. No typhoid organisms were isolated from any of the specimens. An organism was found in ten of the specimens, however, which resembled both typhoid and paratyphoid types in some respects, and differed in others.

This organism was obtained from feces of the cook of the Rochester and also from Cases 16 and 21.

It will be seen that the organism is similar to typhoid in that it does not ferment dulcitol and does ferment dextrin. Like *B. paratyphosus* (B) it produces visible amounts of gas and ferments arabinose.

TABLE 1
RESULTS OF CARBOHYDRATE FERMENTATION TESTS OF CULTURES ISOLATED FROM FECEs

Culture	Dex- trose	Lac- tose	Gas in Dextrose	Malt- ose	Man- nite	Saccha- rose	Radi- nose	Imi- cite	Arabin- ose	Galac- tose	Levu- lose	Dex- trin	Glyc- erin	Inu- lin	Sali- cin	Gela- tin	Indol	Milk
Typoid.....	+	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	-	+
Paratyphoid (A)	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	+
Paratyphoid (B)	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	+
13.2.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
15.1.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
16.3.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
21.14.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
22.4.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
24.1.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
30.6.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
33.29.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
45.1.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
54.2.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+
58.2.....	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+

Integral numbers of the cultures refer to the number of the case; the decimal refers to the number of the culture from the case. The sign + indicates the production of acid except in the column indicating gas production. In the column under gelatin, -- signifies no liquefaction during sixty-three days.

It is about the size of the typhoid bacillus, actively motile, and is gram-negative. According to its cultural characters and carbohydrate reactions, this organism would be classified as a member of the intermediate group. It could not be reckoned as a true gaertner organism on account of its production of permanent acidity in litmus milk. Evidently, it is one of the paragaertner organisms.

SERUM REACTIONS.—These cultures were tested with the serum of the patients from whom they were isolated, as well as with known typhoid and paratyphoid (A) and (B) sera. No reaction was obtained except in the case of the cook (Case 33). Cultures obtained from him agglutinated with his serum at a dilution of 1:60. Two rabbits were immunized against Culture 16.3 obtained from a fatal case. Five inoculations of 1 c.c. of killed twenty-four-hour broth cultures at intervals of 7 days in one rabbit and 5 days in the other failed to produce a serum reacting strongly with the organism injected or any of the other organisms isolated during the epidemic. One of the sera did produce an agglutination at a dilution of 1:10. Both sera, however, gave a positive reaction with *B. typhosus* at a dilution of 1:60. This would account for the positive agglutination tests among those of the party who were infected. The organism is evidently one which stimulates the formation of agglutinins slowly. Those patients who did not give positive agglutination tests had probably not been infected long enough.

PATHOGENICITY FOR LABORATORY ANIMALS.—The pathogenicity of the different strains evidently varied. Each strain was inoculated into a mouse but several of them did not succumb. Culture 16.3 isolated from one of the fatal cases seemed to be most pathogenic.

Mouse 2.—Weight, 20 gm. Inoculated subcutaneously with 0.2 c.c. twenty-four-hour broth culture of 16.3; death in 12-18 hours; congestion about point of inoculation, lungs somewhat congested. Organism isolated in pure culture from liver, lungs, heart, and kidneys.

Mouse 21.—Weight, 25 gm. Inoculated subcutaneously with 0.25 c.c. twenty-four-hour broth culture of 16.3; evidently sick and feverish after forty-eight hours; diarrhea; died after fifty-five hours; lymph glands hemorrhagic, congestion at point of inoculation. Isolated organism from heart, liver, lung, stomach, and feces.

Mouse 12.—Jan. 20, fed bread soaked in twenty-four-hour broth culture of 16.3; Jan. 26, fed bread soaked in twenty-four-hour broth culture 16.3; Jan. 30, drank about 3 c.c. of twelve-hour-broth culture; Feb. 3, drank about 1 c.c. of twenty-four-hour broth culture; Feb. 6, looked sick; Feb. 7, died, eight days after drinking broth culture. Axillary and lumbar lymph glands swollen and

congested, gall-bladder very full, spleen apparently normal, diarrhea. Serum would not agglutinate culture. Organisms isolated from gall-bladder, small intestine, urinary bladder, and kidney. Heart and liver sterile.

Mouse 15.—Drank 2 drops twenty-four-hour broth culture of 16.3; died ten days later; appearance of organs normal. Organism isolated from heart and liver.

Guinea-pig 15.—Drank 2 c.c. twelve-hour broth culture of 16.3; died six days later; gall-bladder much enlarged, suprarenals enlarged, abundant peritoneal fluid, spleen normal, other organs apparently normal. Organism isolated from heart, liver, kidney, peritoneal fluid, gall-bladder, urinary bladder, and small intestine.

Guinea-pig 16.—Weight, 305 gm. Inoculated intraperitoneally, 1 c.c., twelve-hour broth culture of 16.3; died four days later; abscess at point of inoculation, peritoneal cavity full of a white fluid, intestine and mesenteries inflamed, spleen apparently normal. Organism isolated from heart, liver, and peritoneal fluid.

Mouse 7.—Weight, 22 gm. Inoculated subcutaneously with 0.25 c.c. twenty-four-hour culture of 54.2; died after five days; congestion at point of inoculation; lymph glands swollen. Organism isolated from heart, liver, lungs, stomach, large intestine, and point of inoculation.

Mouse 14.—Drank 0.5 c.c. twenty-four-hour broth culture of 54.2; a week later refused to eat, evidently sick; died next day; appearance of organs normal. Organism isolated from stomach, small intestine, and large intestine. Heart, liver, and kidneys sterile.

Mouse 6.—Weight 22 gm. Inoculated subcutaneously with 0.25 c.c. twenty-four-hour culture of 33.29; died after fifteen days; spleen swollen; point of inoculation congested. Organism could be isolated only from point of inoculation.

Mouse 19.—Weight 20 gm. Inoculated subcutaneously with 0.2 c.c. twenty-four-hour broth culture of *B. paratyphosus* (B); died in forty hours; appearance of organs normal; lymph nodes congested; mesenteries congested. Organism isolated from heart, liver, kidneys, small intestine, and large intestine.

Examination of the organs in fatal cases showed no marked pathological changes. When infection occurs through the digestive tract, it is capable of producing enteritis and diarrhea in mice and guinea-pigs.

Mouse 12 was apparently unaffected ten days after eating solid food infected with culture of 16.3 but died on the eighth day after taking the organism in a liquid. Two other mice (Mice 13 and 15) died seven and ten days, respectively, after drinking liquid culture. Infection through the intestine is best accomplished by means of liquid rather than solid food.

PROPHYLACTIC TREATMENT OF MEMBERS OF THE PARTY

Sixteen members of the party had taken typhoid immunizing treatment less than a year before this epidemic. Of these, five were affected with enteritis and diarrhea and one (Case 40) was considered at the

hospital as having typhoid. Two other cases considered as typhoid fever (one of them Case 21) gave definite histories of having had it before. In view of the opinion among medical men that one attack of typhoid protects against another and that the prophylactic treatment protects for at least two years, these data would confirm our findings that it was not the typhoid bacillus with which the members of the party were infected.

SUMMARY

An epidemic has occurred resembling in many ways a water-borne typhoid epidemic. A careful bacteriological examination of feces of patients was negative for typhoid, but an organism was found which seemed to have the cultural characteristics of the paragaertner group.

COMPLEMENT FIXATION IN ACUTE RHINITIS *

KATHARINE HOWELL

(From the Memorial Institute for Infectious Diseases, Chicago)

Tunncliffe¹ discovered in the early stages of acute rhinitis an anaerobic bacillary or spirochetal organism in the nose which has been designated tentatively as *Bacillus rhinitis*. The organism produced acute coryza experimentally in the human subject, and in the early stages of the experimental, as well as of the natural, disease Tunncliffe found the opsonin for the organism to be below normal, the index rising as the infection subsided. In view of these results it seemed of interest to determine whether the sera of cases of acute rhinitis contain any complement-fixing antibodies for this organism.

METHOD

The following bacteria were used as antigens: *Bacillus rhinitis*, suspensions of either single strains or of a mixture of six strains; *B. fusiformis*; *staphylococcus*; *streptococcus*, hemolytic and viridans; *pneumococcus*; *Micrococcus catarrhalis*; *B. influenzae*; *B. mucosus*; diphtheroid bacilli. The strains of *B. rhinitis* were furnished by Dr Tunncliffe.

The strains of the *bacillus rhinitis* and of the fusiform bacillus were grown anaerobically for forty-eight to seventy-two hours on goat blood agar or in ascites broth. The other bacteria were grown aerobically for twenty-four hours on agar slants. In each case, suspensions were made in salt solution and heated at 56 C. for one hour.

The anticomplementary unit of each antigen was determined carefully and in the tests $\frac{1}{2}$ to $\frac{1}{16}$ of this unit was used.

The sera from the patients were heated to 56 C. for thirty minutes and 0.02 c.c. of the undiluted serum constituted the unit.

The antisheep hemolytic system was used, 0.1 c.c. of a dilution 1:10 of fresh guinea-pig serum being the complement unit, and the quantity of sheep blood used was 0.1 c.c. of a 5 percent suspension. The amboceptor was titrated separately for each series of tests, and twice the hemolytic unit was used in each test. Whenever natural

* Received for publication April 1, 1915.

1. Jour. Infect. Dis., 1913, 13, p. 283.

antisheep amboceptor was encountered in the serum to be tested, amboceptor enough to make two hemolytic units only was added.

The serum, the antigen, the amboceptor, the complement, and the sheep blood and the hemolytic system were controlled in each series of tests.

In making the tests the antigen, the serum to be tested, and the complement were incubated at 37 C. for one hour. The amboceptor and corpuscles were then added and the mixture incubated for thirty minutes or longer according to the controls.

THE SERA OF PERSONS INOCULATED WITH THE BACILLUS RHINITIS

The sera of three persons each of whom had received several doses of autogenous vaccines of the bacillus rhinitis were available. In each case the serum caused fixation of complement with the autogenous antigen and a less marked positive result with the other two antigens of the bacillus rhinitis and with the polyvalent rhinitis antigen. It was possible to make frequent tests of the serum of one of these persons in whom the inoculations were discontinued in the middle of November, 1914. At this time, the serum gave a markedly positive result with autogenous antigen. The fixation became less and less marked and in February, 1915, there was no fixation. Three inoculations of vaccines were now made, and the serum again gave a strongly positive reaction.

TABLE 1

COMPLEMENT FIXATION BY THE SERA OF PATIENTS INOCULATED WITH BACILLUS RHINITIS

Serum	Antigen	Results
Case 1.....	B. rhinitis 1.....	++ +
	B. rhinitis 2.....	++
	B. rhinitis 3.....	+
	B. rhinitis, polyvalent.....	+
	B. fusiformis.....	0
Case 2.....	B. rhinitis 1.....	++
	B. rhinitis 2.....	++
	B. rhinitis 3.....	++
	B. rhinitis, polyvalent.....	++
	B. fusiformis.....	0
Case 3.....	B. rhinitis 1.....	+
	B. rhinitis 2.....	+
	B. rhinitis 3.....	++
	B. fusiformis.....	0

B. rhinitis 1 was originally isolated from Case 1; B. rhinitis 2 from Case 2; B. rhinitis 3 from Case 3.

The sign ++ = complete inhibition; + = partial inhibition; + = slight inhibition; 0 = no inhibition.

In these tests *B. fusiformis* antigen was included in each case because of the resemblance between this organism and *B. rhinitis*. The results, however, indicate no relationship.

SERA OF PATIENTS WITH ACUTE RHINITIS (ACUTE COLDS)

The sera of nineteen cases of acute rhinitis have been examined. In most cases several strains of the bacillus rhinitis were used as antigens. There were slight variations in results with different strains, but in no case did the serum give a positive reaction with one or more strains and a negative reaction with any of the other strains. Of the nineteen cases studied, seven gave strongly positive results during some stage of the attack; four less strongly positive; and six only slightly positive. The remaining two cases gave negative results. It was difficult to obtain exact information regarding the duration of the attack when serum was obtained. However, the strongly positive sera were obtained as a rule between the third and eighth days of the attack. During the earliest stages the serum usually caused no fixation, or only slight fixation, of complement. The results in the cases in which serum was obtained several times indicate that the period during which the serum will cause fixation with the bacillus rhinitis as antigen is rather short. In one case the serum remained positive for six weeks and in another case for two weeks, but usually the power of fixation was lost within a few days. In five instances the serum had been examined before acute rhinitis developed, with negative results. During the attack the serum in two cases became strongly positive, one a little less strongly positive, and two feebly positive.

The sera of three patients with acute rhinitis giving positive fixation with the bacillus rhinitis were tested also with the other antigens mentioned in describing the method used in this work, and one serum gave a slight fixation with the pneumococcus antigen, altho there were no pneumococci present in cultures made from the nose. The patient had had pneumonia two years previously. Two of the sera gave a feeble or slight fixation with pseudodiphtheria antigen; one of these two sera gave an equally positive result before the cold. In eight cases in which complement fixation was obtained with rhinitis antigen, tests were made also with antigens of whatever other bacteria were found in smears or cultures from the nose, but with negative results.

THE SERA OF NORMAL PERSONS AND OF PATIENTS WITH DISEASES
OTHER THAN RHINITIS

Tests were made for complement fixation with the bacillus rhinitis as antigen with the sera of thirteen persons apparently in normal condition and without any history of a recent attack of rhinitis. In each

case the result was frankly negative. The sera of patients with scarlet fever, typhoid fever, pneumonia, rheumatism, diphtheria, Vincent's angina, measles, and syphilis were tested in a similar way with negative results in every case except one case of measles which gave a feebly positive result.

TABLE 2
COMPLEMENT FIXATION BY THE SERA OF PATIENTS WITH ACUTE RHINITIS

Number	Approximate Duration in Days of Attack	Results
1	3	+++
2	7	+
3	3	+++
4	3	+++
5	2	+
6	Several	+++
7	Several weeks	+
8	2	0
9	3	+
10	Several	++
11	Several	++
12	Repeated attacks	++
13	2	+
14	2	+
14	4	+++
15	2	+
16	2	0
16	4	+
16	5	0
17	2	+++
18	4	++
18	8	+++
19	2	0
19	4	0

The sign +++ = complete inhibition; ++ = partial inhibition; + = slight inhibition; 0 = no inhibition.

TABLE 3
COMPLEMENT FIXATION BY THE SERA OF PATIENTS WITH ACUTE RHINITIS IN THE PRESENCE OF VARIOUS ANTIGENS

Case	Antigens									
	B. Rhinitis	Pneumococci	Staphylococci	Streptococcus Hemolytic	Streptococcus Viridans	B. Mucosus	B. Fusiformis	B. Influenzae	M. Cattarrhalis	Pseudo-Diphtheria Bacillus
1	+++	0	0	0	0	0	0	0	0	+
2	++	+	0	0	0	0	0	0	0	0
3	++	0	0	0	0	0	0	0	0	+
4	+++	+++
5	+++	0
6	+	0	0
7	+++	0	0	0	0	0
8	+	0	0	0
9	0	0	0	0
10	+	0	0	0	0	0	0

The sign +++ = complete inhibition; ++ = partial inhibition; + = slight inhibition; 0 = no inhibition.

CONCLUSIONS

With the use of the bacillus rhinitis as antigen, fixation of complement is obtained with the sera of persons with acute rhinitis and of persons injected with the bacillus after it is killed by heat.

The fixation is most marked a few days after the onset of the infection and lasts only a short time.

Sera of normal persons and of patients with various infectious diseases do not give complement fixation with the bacillus rhinitis.

The sera of patients with acute rhinitis do not give fixation of complement, except occasionally when suspensions of various bacteria (pneumococci, staphylococci, streptococci, influenza bacillus, fusiform bacillus, pseudodiphtheric bacilli, etc.) ordinarily regarded as closely associated with rhinitis, if not the actual cause thereof, are used as antigens.

These results indicate that the bacillus rhinitis bears a specific relationship to acute rhinitis as ordinarily observed in this region.

CONGLUTINATION IN THE DIAGNOSIS OF DOURINE (TRYPANOSOMIASIS OF THE HORSE) *

HEINRICH WEHRBEIN

(From the Veterinary Research Laboratory of Iowa State College, Ames, Iowa)

The preparation of the conglutinating system is explained fully by Stranigg in his article on the diagnosis of glanders.¹ He states that conglutination has been used for the diagnosis of glanders, lues, dysentery, and also for the recognition of different plant albumins.

My experience in working out a good system is on the whole the same as Stranigg's. I would add that it is sometimes difficult to get a powerful ox serum, as there are vast differences in the potency of conglutination of different sera. The best titer in my experience so far is 0.1 c.c. with 0.1 c.c. horse serum as complement and 0.1 c.c. of a 5 percent emulsion of sheep blood. It is important to know that in older samples of ox serum a sediment sometimes is formed; such sera give stronger hemolytic than conglutinating action, hence cannot be used. For this same reason, ox serum can not be kept mixed with salt solution. I used well-preserved serum for two and one-half months without noticing a change of the titer.

The complement in horse serum is very sensitive and loses most of its activity after six or seven hours, but it has this advantage, that it always has the same titer, provided the horse used is in a normal condition. It is well to select the best serum of about fifteen, as it is very important to have a reliable complement. Some sera agglutinate very strongly; for example, the serum of a sixteen-year-old mare agglutinated down to 0.07 c.c. The average titer of the horse complement is 0.05 c.c. The best titer I have obtained is 0.02 c.c. Sera which agglutinate so strongly that the serum control (0.1 c.c.) is not negative, of course should not be used.

The emulsion of sheep blood should have the same density always; therefore Stranigg titrates it each time. It is easier and quicker to find the density with the blood-count apparatus. In my experiments, using 0.1 c.c. of a 5 percent emulsion, I found that the density

* Received for publication April 2, 1915.

1. Arch. f. Wissensch. u. prakt. Tierheilk., 14, 30, p. 166.

could vary between 700,000 and 900,000 corpuscles without affecting the result. The emulsion can be used for seven days.

As antigen, I used pure trypanosomes and an emulsion of the spleen of a rat which had died of trypanosomiasis (*tr. equiperdum*); but the latter as antigen proved unsatisfactory. For the preparation of the trypanosome emulsion, I inoculated about twenty white rats and bled

TABLE 1
TITRATION OF OX SERUM

Number of Tube	Salt Solution	Fresh Horse Serum as Complement	Inactivated Ox Serum	Sheep Blood Emulsion (5%)
1	0.75	0.1	0.15	0.1
2	0.8	0.1	0.1	0.1
3	0.15	0.1	0.075 = 0.75	0.1
4	0.4	0.1	0.05 = 0.5	0.1
5	0.65	0.1	0.025 = 0.25	0.1
6	0.8	0.1	0.01 = 0.1	0.1
7	0.9	0.1	0.1
8	0.9	0.1	0.1
9	1.0	0.1

Three hours at 37 C.

Twice the smallest amount giving complete conglutination is used as titer.

TABLE 2
TITRATION OF COMPLEMENT

Number of Tube	Salt Solution	Fresh Horse Serum	Inactivated Ox Serum	Suspension of Sheep Blood
1	0.8	0.1	×	0.1
2	0.09 = 0.9	×	0.1
3	0.1	0.08 = 0.8	×	0.1
4	0.2	0.07 = 0.7	×	0.1
5	0.3	0.06 = 0.6	×	0.1
6	0.4	0.05 = 0.5	×	0.1
7	0.5	0.04 = 0.4	×	0.1
8	0.6	0.03 = 0.3	×	0.1
9	0.7	0.02 = 0.2	×	0.1
10	0.8	0.01 = 0.1	×	0.1
11	0.9	0.1	0.1
12	0.9	×	0.1
13	0.85	0.1	×/2	0.1
14	1.0	0.1

The result is read after three hours at 37 C.

The smallest amount of horse serum giving complete conglutination is the titer.

as many of them after two to three days as proved to be heavily infected. The blood was mixed with 1 percent citrate solution and centrifugalized. The trypanosomes, which constitute the superficial layer of the sediment, are then separated with the pipette, the process being repeated as often as necessary. In addition the trypanosomes are thoroughly washed, as it is important to remove all citrate and rat serum,

because these substances hinder the conglutination, even tho only a minute amount is present. The density of the final emulsion may be 1:100. I append the different titration tables. Usually I read the reactions three hours after I add the ox serum and the emulsion of sheep blood. I cannot convince myself that it is an advantage to wait eight hours, as Stranigg does.

The antigen acts similarly as in the usual complement-fixation; the presence of the horse serum decreases the anti-complementary action of the antigen even to a higher degree. Therefore, it is best always to titrate it with the positive and negative serum. One can use the antigen about two weeks, if it is clean, and it is sufficient to titrate it every third day.

TABLE 3
TITRATION OF ANTIGEN

Number of Tube	Salt Solution	Complement	Serum	Antigen
1	To make 1 c.c.	As determined by titration.	Dourine S. 0.1	0.02
2			Dourine S. 0.1	0.05
3			Dourine S. 0.1	0.1
4			Dourine S. 0.1	0.15
5			Dourine S. 0.1	0.2
6			Dourine S. 0.1	0.25
7			Dourine S. 0.1	0.3
8			Dourine S. 0.1	0.35
9			Dourine S. 0.1	0.4
10			Dourine S. 0.1
11			Normal S. 0.1	0.1
12			Normal S. 0.1	0.2
13			Normal S. 0.1	0.3
14			Normal S. 0.1	0.4
15			Normal S. 0.1

After one hour at 37 C. add ox serum and emulsion of sheep blood.
The result is read after three hours at 37 C.

The test serum must be absolutely clean; chemicals and decomposition prevent a satisfactory result and a serum control is always necessary. I inactivate the sera thirty minutes at 59 C.

The reading of the tests, whether conglutinated or not, is very easy, if one has a good system. In the negative tubes, the blood corpuscles appear clumped; in the positive ones, no trace of conglutination is visible.

I have made experiments with nineteen dourine sera, which I hereby tabulate. With the one exception, the results are the same as with complement-fixation, and in some cases, the same as with agglutination.

Two of the thirty normal sera which I tested gave a doubtful result. One of them gave a positive reaction four times, the serum control

showing a partial imbibition. Complement-fixation repeated several times gave absolutely negative results. The agglutination test was doubtful. One other serum gave the same trouble, with the exception that the serum control was not questionable and an agglutination test was not made. Both sera were sent to the laboratory so that clinical observation was not possible.

In the beginning of the experiments, I sometimes had positive reactions in the negative row of the antigen titer; it could be shown that this was caused by contaminated antigen. Preserved antigen and sera proved to be unsatisfactory.

One serum of a donkey gave a negative result, the same as the agglutination. Using the complement-fixation method, one regularly gets positive results with donkey sera, which are therefore very unsatisfactory for use. The donkey mentioned was under observation for a long period and ostensibly healthy.

TABULATION OF THE SERA TESTED

Positive Sera

(1) Stallion, three years old, gave positive complement-fixation, July, 1914. At the same time plaques on the right flank; in one of these plaques, trypanosomes were found; good condition. Used as positive control.

(2) Mare, gave positive complement-fixation since February, 1914. Depigmentation on the vulva; plaques on the right flank; mediocre condition. Used as positive control.

(3) Mare, gave positive complement-fixation since February, 1914. Good condition; no clinical symptoms.

(4) Mare, gave positive complement-fixation since 1913. Mediocre condition; weak in the posterior extremities.

(5) Mare, gave positive complement-fixation until March, 1914; in August of the same year gave only conglutination positive, not complement-fixation. Had been infected by cohabitation in September, 1907; in December of the same year, facial paralysis of the left side; complete recovery after atoxyl treatment.

(6) Mare, gave positive complement-fixation in March and August, 1914. Natural infection March, 1909; no clinical symptoms; good condition.

(7-19) Sera of naturally infected horses, without clinical symptoms, gave equally positive complement-fixation and conglutination.

Negative Sera

(1) Gelding, under observation since birth, was used as negative control.

(2) Donkey, seven years old, gave positive complement-fixation. When the serum was treated with 5 percent carbolic acid, it reacted negatively. The untreated serum, however, gave a positive reaction, when pure trypanosomes were used as antigen, or even a normal rat spleen. The donkey was under observation and showed no clinical symptoms. Negative conglutination.

(3-11) Sera of healthy horses under observation.

(12-21) Sera sent in for test. Among these are the ones mentioned as giving doubtful results. One, the serum of a mule, gave positive complement-fixation; negative agglutination. In no case was it possible to make clinical observations.

(22-30) Nine mares, doubtfully infected or ostensibly recovered, gave negative serum reactions.

CONCLUSIONS

The agglutination method can be used for the diagnosis of dourine; but it is more sensitive to faulty technic and hence more difficult to employ than the usual complement-fixation method.

THE FERMENT ACTIVITY OF THE BLOOD SERUM IN INFECTIOUS DISEASES *

FREDERICK HOWARD FALLS

(From the Departments of Pathology and of Obstetrics of the University of Illinois, Chicago)

It has been shown that blood serum under various conditions has the power of splitting proteins into less complex compounds. E. Abderhalden¹ has proved this to be true of the serum of pregnant women, and of that of patients suffering from carcinoma. He is supported and his work has been confirmed by a great many observers, both in Europe and in this country. Fauser,² Flatow,³ and many others have demonstrated ferment activity in the blood in cases of dementia praecox, epilepsy, and other nervous affections.

In this country Williams and Pearce, Jellinghaus and Losee, Schwartz, McCord, and others, report similar results. Jobling, Eggstein, and Petersen⁴ have shown that normal guinea-pig serum contains active tryptic ferments which can be demonstrated, after removing the antitryptic substances, by treating the serum with various agents, such as kaolin, starch, and iodine, and then allowing the serum to act on preparations of casein.

In a recent article I⁵ have shown that by the dialysis method ferments can be detected in the serum in a large number of pathological conditions, as well as during pregnancy, and at the height of digestion; also that apparently normal individuals occasionally give positive reactions. I pointed out that the length of time in the dialyzer has much to do with the occurrence of a positive or negative reaction. S. Kjaergaard⁶ has supported this in a recent article; he also confirms my findings of positive reactions in other conditions, as carcinoma, but he has not reported experiments with sera from infectious diseases. Fränkel⁷ supports my view entirely; he found that sera from various pathological conditions usually gave positive reactions.

* Received for publication April 5, 1915.

1. Abwehrfermente.

2. München. med. Wchnschr., 1914, 61, p. 126.

3. Ibid., p. 1168.

4. Jour. Exper. Med., 1915, 21, p. 239.

5. Jour. Am. Med. Assn., 1914, 63, p. 1172.

6. Ztschr. f. Immunitätsforsch. u. exper. Therap., 1914, 22, p. 31.

7. Ibid., p. 549.

The work now reported was undertaken with the idea of determining what pathological conditions cause an increase in the ferment content of the blood that could be detected by the dialysis method, placenta being used as a substrate. It was decided systematically to investigate a number of conditions, examining enough cases of each to warrant drawing conclusions, in the hope that by this means some light might be shed on the underlying principles of infection and immunity in the various pathological conditions examined.

In investigating a disease, two objects were especially observed: (1) the presence or absence of the ferments as indicated by positive or negative reaction; (2) relative strength of the reaction compared with the stage, and clinical severity of the disease.

Lobar pneumonia was the first disease investigated. There is reason to believe that the crisis in this disease is due to the rapid mobilization of a large amount of powerful ferment in the blood stream. Indeed Dick⁸ has shown that proteolytic ferments develop in the blood during pneumonia, about the time of crisis. He notes also that the ferments seem to have a special action upon pneumococcus protein. In this work, the tryptic power of the serum was determined by the polariscope. I assumed that if these ferments were not absolutely specific, their presence ought to be capable of demonstration by the dialysis method, properly prepared placental tissue being used as a substrate.

The technic followed was that given by Abderhalden,⁹ and inactivated serum plus placenta was used as a control in each case. The controls were uniformly negative. For excellent English descriptions of the technic, reference may be made to articles by H. Schwartz,¹⁰ and Jellinghaus and Losee.¹¹

A few points in technic that gave most trouble in the early part of this work and which have been called to my attention as sources of error by other workers in this field, may be mentioned here:

(a) Apparatus and glassware must be scrupulously clean, physically and chemically.

(b) Blood serum must be fresh, and absolutely free from hemolysis.

(c) Bacterial contamination of the serum must be avoided as much as possible; and the growth of bacteria prevented by generous use of toluene.

(d) Incubation should be allowed to proceed twenty to twenty-four hours instead of sixteen to eighteen hours, as was originally advised by Abderhalden.¹² He now also uses the longer period in his work.

(e) Instead of 1.5 or 2 c.c. of serum, as Abderhalden advocated at first, 1 c.c. of serum is used. He now advises the lesser amount.

8. Jour. Infect. Dis., 1912, 10, p. 383.

9. Beitr. z. Klin. d. Infektionskrank. u. Immunitätsforsch., 1913, 1, p. 243.

10. Am. Jour. Obst., 1914, 69, p. 54.

11. Ibid., p. 593.

12. München. med. Wchnschr., 1914, 61, p. 8.

(f) After each time that the dialyzing thimbles are used, they should be washed scrupulously clean, and allowed to stand several hours in fresh distilled water, so that any digestion products may dialyze out of the tube wall, and all the serum be cleansed away.

(g) The dialyzing thimbles are best kept under water in a large jar and brought to boil just before using. Prolonged boiling is said to alter the permeability of the membranes, rendering them thicker.

(h) The placenta must be obtained fresh.

I start the preparation within a few seconds after the birth of the placenta. The amnion and cord are dissected off rapidly, and the remaining tissue is cut into pieces the size of a hazelnut. These are placed in a piece of gauze about one foot square and two or three layers thick. The edges are folded up to make a bag, and the tissue is washed in cold running water, or in frequently changed water, until the wash water remains absolutely clear. During this process the tissue is constantly kneaded and pressed between the hands. The bag is then opened and any pieces of tissue containing clotted blood are discarded; pieces having a pink tinge are broken up into smaller pieces, and the washing repeated until the tissue appears absolutely free from blood. Now pieces of tissue with the faintest tinge of pink are discarded, and the rest placed in about a liter of boiling water. The washing takes about two to two and one-half hours, and must be uninterrupted. After boiling for five minutes, the water is poured off, and the tissue is washed in several changes of water, being well squeezed by the hands in each to remove all the extracts from the tissues. The tissue is again boiled for five minutes and the washing repeated. This is repeated about six times; then the tissue is boiled with five times its volume of water for five minutes. Next 5 c.c. of the filtrate is tested with 1 c.c. of a 1 percent ninhydrin solution by boiling for one minute. If any violet color is noted in this fluid after standing one-half hour, the boiling and washing should be repeated three or four times. In the event, as sometimes happens, that the filtrate as obtained above still gives the reaction, it is best to discard this tissue, and prepare another placenta. It has been found by others as well as myself that some placentae apparently cannot be freed from substances reacting with ninhydrin, by the method herein described. When the filtrate from the boiled placenta no longer gives the faintest trace of color on boiling with ninhydrin as described, the tissue is boiled once more in fresh distilled water, placed in sterile, colored bottles, and covered with boiling water. It is then cooled to room temperature, and two drams of chloroform added to each eight-ounce bottle. If the chloroform is added before cooling the jars, it will boil away rapidly. Toluene is now added to form a layer about one-eighth of an inch thick on the surface of the fluid. Enough tissue for immediate needs is removed with sterile forceps from the stock bottles before setting up each experiment, and is boiled three times in three changes of water before using in a test. It is well to test 5 c.c. of the filtrate from the last boiling with 1 c.c. of a 1 percent solution of ninhydrin to be sure that the tissue just before going into the test is absolutely free from dialyzable, ninhydrin-reacting substances.

This work when properly done is tedious and time consuming; nor can it be done in bulk. Each test requires individual attention at all stages to insure uniformity, and to avoid errors in the technic. Various modifications in the technic at the present stage of our knowledge are inadvisable, because they render the work of the various investigators impractical for comparison. The technic is not too difficult for any well-trained person to master, but personal attention to each detail of preparation for and carrying out of the test is

necessary, and no part of the work can be entrusted to unskilled assistants, or persons not familiar with the theoretical principles underlying the test. For example, results obtained by men using serum collected by someone else at a distance from the laboratory, and often forty-eight or more hours old when used, are of no possible significance.

DISEASES

Pneumonia.—Nineteen cases of lobar pneumonia were studied. Five cases before the crisis, third to seventh day, were observed. The reaction was negative in one, and weakly positive in another, in which the blood was taken on the third and fourth days respectively, and the dialysis allowed to proceed twenty and twenty-one hours. The reaction was moderately strong in two of the remaining cases, and strong in the third; but in these instances dialysis proceeded twenty-seven hours.

Blood was obtained during lysis in two cases, one of which gave a very strong reaction, the other a moderately strong one.

Blood was obtained also from twelve cases at varying periods after deferescence, from one day to four and one-half weeks. In this group the reactions were very strong in most cases shortly after the crisis, and progressively weaker as convalescence was established. It was negative in one case one week after crisis. The varying strength of reaction and period of dialyzation will be seen in Table 1. Case 6 is especially interesting, because clinically it was an unresolved pneumonia, and, as might be expected theoretically, gave a weak reaction.

TABLE 1
LOBAR PNEUMONIA

Number	Day of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	3	6	20	0	I.S.+P			
2	4	4	21	+ very weakly positive	0	M	25	
3	4	4	27	+++++	0	M	43	
4	5	4	27	+++	0	M	35	
5	7	4	27	+++	0	M	28	
6	30	$\frac{1}{2}$	22	+	0	M	44	Unresolved pneumonia
7	During lysis	12	21	+++++	0	M	25	
8	During lysis	5	25	+++	0	M	29	Still has fever due to pleurisy
9	1 (past crisis)	4	25	+++++	0	M	35	
10	10 (past crisis)	12	24	+++	0	M	44	
11	21	4	24	+++++	0	M	53	Lysis
12	11 (past crisis)	12	24	+++++	0	M	54	
13	2 (past lysis)	$\frac{1}{2}$	24	+++++	0	M	33	
14	3 (past lysis)	12	20	Weak +	0	M	22	
15	8 (past crisis)	$2\frac{1}{2}$	16	+ weak	0	M	33	
16	2 (past crisis)	8	16	++	0	M	22	Slight hemolysis of serum
17	14 (past crisis)	16	+ weak	0	M	20	
18	$1\frac{1}{2}$ (past crisis)	16	+++++	0	M	23	
19	7 (past crisis)	16	0	0	M	19	

It would appear from these results that during an attack of pneumonia, the ferment content of the blood is increased. This increase is especially noted during, or just after, the crisis, and rapidly disappears in the convalescence.

This corresponds very well with our clinical knowledge of the disease, and with its short-lived immunity. It coincides also with the view I advanced in a previous paper as to the probable source of the ferment in this disease. The source is probably the consolidated lung, which in the stage of gray hepatization contains an enormous amount of pure ferment. Osler states that, as far as can be demonstrated by physical signs, the lung may be clear by the eighth day after crisis.

Recently Jobling, Eggstein, and Petersen¹³ have demonstrated an increase in the antitryptic power of the blood serum in cases of pneumonia, tuberculosis, and pregnancy. This work is supported by that of Plaut,¹⁴ who obtained positive Abderhalden tests with various inorganic absorbing substances, as kaolin, talc, barium sulphid, and infusorial earth.

Malaria.—Fourteen cases of malaria were studied, thirteen of which were of the tertian type and one of the estivo-autumnal type. They were taken at various stages of the disease. Most of the cases were studied after the cessation of the chills, following the administration of quinin. Every case gave a positive reaction, but great variation in intensity of reaction was noted. At the beginning of the work it was thought that shortly after a chill, with the rupture of many red blood cells, a demonstrable increase in the ferment content of the blood might be detected. This surmise was not borne out by the results as shown in Table 2. From a study of these figures, it would appear that shortly

TABLE 2
MALARIA

Number	Duration of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	5	24	++++	0	M	52	Tertian
2	2 months.....	5	23	++++	0	M	58	Autumnal
3	10 days.....	4	23	+	0	M	57	Tertian
4	Last chill 3 days before	5	23	+	0	M	43	Tertian
5	Last chill 2 days before	5	23	+	0	M	17	Tertian
6	3 weeks. Last chill 5 days before	4	23	+	0	M	51	Tertian
7	1 month. Last chill 7 days before	4	25	++++	0	M	41	Tertian
8	4 weeks.....	5	..	+++	0	M	44	Tertian
9	3 weeks. Last chill 4 days before	5	23	+++	0	M	34	Tertian
10	11 days. Last chill 3 days before	5	23	+	0	M	..	Tertian
11	9 days. Last chill 1 hour before	4	25	++	0	M	35	Tertian
12	15 days. Last chill 24 hours before	4	25	++++	0	M	36	Tertian, no quinin
13	18 days. Last chill 10 days before	4	23	++++	0	M	26	Quinin
14	1 week. Last chill 18 hours before	4	23	+++	0	M	17	Hemolyzed serum
15	8 hours.....	5	28	+	0	M	20	

13. Jour. Exper. Med., 1915, 21, p. 239.

14. München. med. Wchnschr., 1914, 61, p. 238.

after a chill there is a rather large amount of ferment in the blood. After quinin administration, and control of the chills, a period follows in which the blood is relatively poor in ferment. When convalescence is fully established, and the patient apparently well, the ferment content of the blood increases markedly. Quinin appears to have no inhibitory effect on the action of the ferments themselves, since cases taking large amounts often gave strong reactions.

Acute Articular Rheumatism.—The cases examined were in various stages of the disease, and the diagnosis was based on careful physical examination, together with a complete history of the case. No doubtful cases of possible gonorrheal or septic arthritis were used.

TABLE 3
ACUTE ARTICULAR RHEUMATISM

Number	Duration of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	2 weeks.....	5	22	++	0	M	55	Light attack; no fever
2	4 weeks.....	5	22	+++	0	M	21	No fever
3	2 weeks.....	4	22	+ faint	0	M	38	Temp. 101
4	4 weeks.....	5	22	+	0	M	28	Temperature 100 three days previously
5	3 weeks.....	5	22	+ faint	0	M	35	Temp. 101
6	4	18	+++	0	M	14	Mitral regurgitation
7	6 weeks.....	5	22	0	0	M	27	Two glasses milk 2½ hours and ½ hr. prev.
8	5	18	++	0	M	25	No fever for several days

The results are shown in Table 3. From a study of this, it would seem that in general the reaction is relatively weak during the early stage of the disease while the patient is feverish, and gradually increases in intensity during the period of convalescence when the case is progressing favorably. However, enough cases have not been examined to determine the prognostic value of this phenomenon. It is probable that the reaction gets weaker, or entirely disappears, after complete recovery from the infection, as is suggested by Case 7. This would also coincide with our clinical knowledge regarding the short period of immunity following an attack of acute articular rheumatism.

Until more extensive studies are made, it is impossible to state whether this mobilization of ferment is to be regarded as an etiologic factor, or merely as a concomitant factor in the development of an immunity to this disease.

Typhoid Fever.—Ten cases of typhoid fever were studied. These were all carefully selected cases, each giving either positive agglutination tests, or a positive blood culture. The results obtained (Table 4) are somewhat difficult to interpret. The reaction was positive in every case, but the variation in intensity seemed to follow no definite variation in the clinical course of the disease. The series is too short to permit any definite conclusions, but it appears probable from the results that in the second week of the disease the ferments are increased to a considerable extent, and that this increase gradually

TABLE 4
TYPHOID FEVER

Number	Duration of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	9 days.....	5	23	+	0	M	18	
2	8 weeks.....	4	22	++++	0	M	19	Recurdence second week
3	10 days.....	7	22	+++	0	M	13	Very toxic
4	6½ weeks.....	5	23	++	0	M	27	
5	6 weeks.....	4	22	+++	Sl. color	M	21	Control serum heated only to 50 C.
6	2 weeks.....	5	23	Trace	0	M	24	
7	5 weeks.....	5	22	+	Sl. color	M	38	
8	1 week.....	4	27	++++	0	M	25	
9	5 weeks.....	8	24	++++	0	M	36	Very toxic
10	6 weeks.....	4	24	+	0	M	30	

disappears in the later weeks of the disease, except possibly in the very toxic cases.

Meningitis.—Meningitis was next investigated, and it was found that in this condition the blood was relatively poor in ferment. This is as might be expected, as the inflammatory reaction is taking place chiefly outside the blood stream, and hence the foreign proteins in the form of bacteria and their toxic products are not present in the blood in great numbers. Hence we might expect to find few ferments mobilized against them in the blood serum. This can be seen by a study of Table 5.

TABLE 5
MENINGITIS

Number	Duration of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	?	Several	22	Negative	0	M	37	Semicomatose
2	?	3½	22	Faint trace	0	M	50	Pneumococcal or epidemic
3	?	Several	24	Negative	0	M	25	Epidemic
4	18 days	4	21	Faint trace	0	M	20	Tuberculous
5	3½	22	Negative	0	M	30	Wassermann +

Of the five cases studied two were tuberculous, one of which gave a faint positive reaction. This slight reaction might have been due to a focus of tuberculosis elsewhere in the body, as I have found that tuberculosis of the lungs gives a faint positive reaction in the majority of cases. One of epidemic meningitis gave a faint positive reaction, but this case had a concomitant chronic nephritis. One epidemic, and one syphilitic, meningitis gave absolutely negative results.

Pulmonary Tuberculosis.—Of six cases studied, a very weak reaction was obtained in four, and a negative reaction in two cases, as shown in Table 6. The negative reactions occurred in cases of a very chronic type, not very toxic, and without fever. In all, however, tubercle bacilli had been found in the sputum before the cases were used in this series. It is probable that the

positive reaction in the more rapidly advancing cases may be explained, in part at least, on the basis of a mixed infection. Whether this reaction will prove to be of value as a means of prognosis in a given case cannot be determined by this short series of cases; further work is necessary to determine this point.

TABLE 6
TUBERCULOSIS

Number	Duration of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	6 weeks.....	4	19½	Trace	0	M	29	Acute pneumonic phthisis
2	6 months.....	4	19½	Trace	0	M	40	No fever
3	1 year.....	4½	19½	Negative	0	M	46	No fever
4	1 year.....	5	24	Negative	0	M	25	Very chronic; no fever
5	6 months.....	5	24	Trace	0	M	46	Rapid course. Temp. 101
6	2 months.....	5	24	Trace	0	M	..	Acute progressive. Temperature 101

Tabes Dorsalis and General Paresis.—These diseases were studied because they represent a different type of infection from the other groups. The cases were in various stages, and some were treated while others were not. The cases were all in adult men with the exception of one case of congenital tabes in a boy of fifteen. All specimens of blood were taken in the same way, and the periods of dialyzation were as nearly equal as possible, with the exception of the first three cases of tabes.

TABLE 7
TABES DORSALIS

Number	Duration of Disease	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	2 years.....	5	20	++++	0	M	26	Stood at room temperature 28 hours
2	2 months.....	5	20	++++	0	M	34	Stood at room temperature 28 hours
3	4 months.....	5	20	+++++	0	M	35	Stood at room temperature 28 hours
4	1 year.....	4	24	+	0	M	52	
5	4	24	++	0	M	55	
6	3 years.....	4	24	0	0	M	46	Slight hemolysis
7	3-4 years.....	6	23	Very faint	0	M	40	Gastric crisis
8	4 months.....	4	23	++	0	M	25	
9	1 year.....	4	23	+	0	M	38	

The results are shown in Tables 7 and 8. The reaction is positive in fourteen of fifteen cases. The reactions were relatively stronger in the cases of general paresis than in the cases of tabes. The first three cases of tabes are not comparable with the other cases, because the serum was dialyzed twenty hours in the incubator and then placed twenty-four hours at room temperature,

before the dialyzation was interrupted. Therefore, the reactions were stronger than those of the other cases of tabes, as might be expected, and approached those of the general paresis cases. This corresponds with our clinical knowledge concerning these diseases. General paresis is a rapidly advancing disease, and the destructive processes are going on rapidly. The duration of the disease is relatively short. In tabes we are dealing with an essentially slow process, and destructive processes are advancing very slowly. In the active process, we should expect the mobilization of a rather large amount of ferment, while in the relatively quiescent process, we should expect relatively little of the ferment to be mobilized in excess of the normal. This is apparently what happens in these conditions. The effect of treatment could not be judged in this short series.

TABLE 8

PARESIS

Num- ber	Time Since Last Meal in Hours	Time of Incuba- tion in Hours	Ninhydrin Reaction	Control Reaction	Sex	Age	Remarks
1	3½	22	+	0	M	33	Doing well under salvarsan
2	3	22	++++	0	M	35	
3	3	22	++++	0	M	42	
4	5	24	++++	0	M	44	Cerebrospinal syphilis
5	3	22	++++	0	M	15	Congenital tabes. Optic atrophy
6	4	22	++++	+	M	34	Taboparesis

TABLE 9

CASES OF PREGNANCY

Num- ber	Period of Gesta- tion in Months	Time Since Last Meal in Hours	Time of Incuba- tion in Hours	Ninhydrin Reaction	Control Reaction	Remarks
1	? 5	5	22	+++	I.S+P 0	Clinical examination con- firms diagnosis
2	9¼	4	16	+++	S 0	
3	9¾	5.	16	+++	I.S+P 0	
4	10	4	16	+++	S 0	Placenta previa
5	9¼	4	16	+++	S 0	
6	9¾	5	16	++	S 0	
7	8¾	4	16	++	S 0	
8	9	4	16	++	S 0	Threatened abortion
9	5	5	22	+++++	I.S+P 0	
10	4	5	21	++++	I.S+P 0	

I have incorporated in this paper a series of cases of pregnancy and of the puerperal state so that a means of comparison might be at hand to judge the strength of the ferment activity in these conditions, as compared with that in the various diseases considered in this paper. These results are shown in Tables 9 and 10, respectively. These tests were made under the same conditions as the tests in the infectious diseases. The same tissue was used as a

substrate, in the same amounts, and, as nearly as possible, the period of dialysis was the same. The reactions were fully as strong, or even stronger, in some of the cases of infectious disease as in the strongest case of pregnancy. However, it will be noticed that there is a more uniform intensity in the cases of pregnancy. This may be explained on the assumption that in healthy women at about the same period of gestation, about the same amount of ferment is mobilized. It is possible also, as I have previously pointed out, that the source of ferment may be different in pregnancy from that in the other conditions. It seems probable that the source of the ferment in pregnancy may be at least two-fold. The ferment may be, as Abderhalden teaches, mobilized by the body to break down and destroy the protein of the placental villi that break off, and are found in the blood stream. Or it may be, as I have said before, that the

TABLE 10
PUERPERAL CASES

Number	Length of Time After Parturition	Time Since Last Meal in Hours	Time of Incubation in Hours	Ninhydrin Reaction	Control Reaction	Remarks
1	Just after delivery	10	22	++++	S 0	Wassermann +
2	8 hours.....	5	22	+	I.S.+P. 0	
3	1½ days.....	5	22	+++	I.S.+P. 0	
4	3 days.....	5	22	++	I.S.+P. 0	
5	3 days.....	3 (liquid)	22	++	S 0	Postpartum eclampsia
6	7 days.....	4	22	++	S 0	Acute puerperal mania
7	5 days.....	5	22	++	I.S.+P. 0	Nursing baby
8	6½ days.....	5	22	+++	I.S.+P. 0	
9	8 days.....	5	22	+++	I.S.+P. 0	
10	13 weeks.....	4½	24	++	I.S.+P. 0	

placenta, which is a loose structure very rich in ferment and engaged in the later months of pregnancy in an immense amount of metabolic activity, both anabolic and catabolic, cannot contain all of the ferments within itself. Hence a leakage occurs of ferment into the maternal blood. These considerations may explain, in part at least, the regularity with which the increase in ferments can be demonstrated in pregnancy, and also the relative constancy of the reaction as to intensity in normal women at the same period of gestation. Experiments are now in progress whereby it is hoped that some more definite light may be thrown on this point.

From a study of these results as a whole, it seems clear that we must regard the proteolytic ferments of the blood as being raised above the normal in nearly all cases of infectious diseases. It seems probable also that this mobilization of ferments takes place in response to certain definite conditions that arise in the blood stream during the course of an infection. As has been shown by many authors, there

is a mobilization of ferments in the blood of all animals after the parenteral injection of a foreign protein. This phenomenon seems to be in the nature of a protective reaction of the body against the possible harmful effects that might arise from the toxic radicle which forms part of every protein molecule. According to Vaughan¹⁵ and many other authors, in cases of infectious disease we must assume that we have a condition in which the body is constantly being invaded by particles of foreign protein, and that this protein in the shape of bacteria, and their metabolic products, is in the blood stream, as well as in the tissues. It is logical to assume that, as a means of defense against this invasion, the body mobilizes ferments that have the power to break down the protein to the end products of tryptic digestion, which are powerless to cause harm in the body.

If, then, these ferments are mobilized in these conditions, they should be capable of demonstration by the Abderhalden dialysis method, and also by the antitrypsin determination method. This has already been done by Jobling, Eggstein, and Petersen¹⁶ and by several others for some of the diseases that I have considered in this paper.

It appears to me that the demonstration of the ferments of the blood by the antitrypsin method and by the Abderhalden dialysis method depend on one and the same thing: an increase in the tryptic power of the blood serum above the normal.

It has long been known that if the trypsin content of the blood be artificially increased by the injection of solutions of trypsin, the antitrypsin in the blood also is increased in direct proportion. Wells¹⁷ cites Hildebrand as having first described this phenomenon, and as pointing out that it depended upon the same principle as the production of immunity in animals by the injection of various proteins. Von Dungern¹⁸ immunized animals against bacteria, and obtained an immune serum against their proteolytic ferments.

Therefore it appears that this work fits in with, and corroborates other work. It is merely a new way of demonstrating what has long been thought probable on theoretical grounds. It confirms, by complementing, the work done by the antitrypsin method in these conditions by other authors.

15. Protein Split Products in Relation to Immunity and Disease, Philadelphia and New York, 1913.

16. Jour. Exper. Med., 1915, 21, p. 239.

17. Chemical Pathology, 1907.

18. München. med. Wechnschr., 1898, 45, p. 1040.

One rather interesting point in these results is the fact that the ferment activity of the serum apparently bears no relation to the leukocytic content of the blood from which the serum was derived. Thus it will be seen by examining the tables, that in the cases of malaria, tuberculosis, and typhoid, diseases in which we find a leukopenia, the reaction is often strong; while in meningitis, and in some of the cases of pneumonia, in which leukocytosis is early, and constantly high, the reaction may be weak, or even absent. This speaks against the view that the ferment is derived from the leukocytes, altho it may be that in the diseases which are characterized by a leukopenia, we have to deal with an overdestruction, rather than an underproduction, of the leukocytes. If this were true the apparent discrepancy between the leukocytic and ferment content of the blood might be explained.

I wish to point out in this connection that I do not consider the dialysis method a delicate one. In working with so many variable factors, qualitative reactions must be accepted with a certain degree of reserve, and only accepted as facts when sufficient evidence is acquired by this and other methods to give weight to conclusions drawn from the results obtained.

CONCLUSIONS

In the infectious diseases the ferment content of the blood is increased above the normal in most cases.

This increase is capable of demonstration by the Abderhalden dialysis method.

The variation in intensity of the reaction in the various diseases at different stages of the infection, may prove of value in diagnosis or prognosis; but there are so many variable factors in the carrying out of the test, as described by Abderhalden, that too much must not be expected from the test along these lines.

The increase in antitryptic power of the blood occurs in these diseases, in all probability, in response to the increase in the tryptic content of the serum, which in turn is probably due to the presence of a foreign protein in the blood stream.

Acute infections in which the reaction between the infecting organism and the body defenses takes place outside the blood stream, cause relatively little increase in the ferment content of the blood serum, as measured by this method.

Pregnancy and the puerperal state, together with many other conditions, give reactions that cannot be differentiated from these reactions. This destroys its practical value as a diagnostic measure.

The source of the ferment is probably not to be found in the leukocytes, because the reaction was often strongly positive in cases in which there was a low leukocyte count—malaria, pregnancy, typhoid, general paresis—and frequently weak in conditions in which the leukocyte count was high—meningitis and pneumonia before the crisis.

THE GERMICIDAL EFFECT OF LACTIC ACID IN MILK *

P. G. HEINEMANN

(From the Department of Hygiene and Bacteriology, University of Chicago)

Reference to the germicidal effect of sour milk or buttermilk on pathogenic bacteria is occasionally found in the literature. It has been intimated that lactic-acid-producing bacteria are a protection against infectiousness of contaminated milk, inasmuch as the acid produced by these bacteria is said to destroy pathogenic bacteria. There is however little positive evidence to show whether this assumption is true or not. Earlier work by Barthel,¹ Bassenge,² and Behla³ is contradictory. An investigation by Northrup⁴ as to the fate of typhoid bacilli in milk and in lactose broth, acidified by cultivation of lactic acid bacteria and filtered through a Chamberland filter, seems to show that acid produced by different lactic acid organisms varies in degree of germicidal action. While one strain of "*Bacterium lactis acidii*" destroyed typhoid bacilli when 0.33 percent acid (as lactic acid) was present, other strains, including the *bacillus bulgaricus*, had the same effect only when nearly twice as much acid was present. Krumwiede and Noble⁵ determined the longevity of typhoid bacilli in sour cream. The authors make the following deductions from their work:

The typhoid bacillus is gradually killed in sour cream by the acids produced, the rate of destruction being proportionate to the degree of acidity and the number of typhoid bacilli present. The apparent disappearance of typhoid bacilli in sour cream, where the normal flora is present, is due in part to overgrowth of the typhoid bacillus by these bacteria and the difficulty of finding by our present methods the proportionately few typhoid bacilli remaining.

With a moderate contamination, the typhoid bacilli were killed in about four days. With a heavy contamination, or where initial multiplication has taken place, a longer time may be required. For this reason a clean cream which soured slowly would be more dangerous if contaminated, as an initial multiplication of the typhoid bacilli would occur and a longer time would be required to destroy the bacilli. Whether under ordinary conditions the overgrowth by the bacteria of the cream is a factor in the death of the typhoid bacilli cannot be determined by our present methods.

* Received for publication April 8, 1915.

1. Die Bakteriologie des Meiereiwesens, Leipsig, 1901.

2. Deutsch. med. Wchnschr., 1903, 29, p. 675.

3. Lafar: Handbuch d. Tech. Mykologie.

4. Michigan Agr. Exper. Sta., Technical Buull., 1911, 9.

5. Am. Jour. of Public Health, 1914, 4, p. 1006.

There are several factors which render this problem difficult of approach. It is known that germicidal agents act differently on bacteria according to the medium in which the action is studied. Not only the substances composing the medium, but the concentration of the substances in the medium, is of importance. We should expect that a certain amount of a germicide in broth, for instance, would act differently on bacteria from the same amount in milk. Milk, therefore, is the only suitable medium for experiments bearing on our subject. But in the use of milk difficulties again are encountered. The growth of saprophytic bacteria in this medium is so prolific that before the lapse of many hours we have to deal with large numbers. Pathogenic bacteria, even if present in numbers large enough to be infectious, might be overlooked by the plating method—the only method available in the present state of bacteriologic technic. We have to consider also that the kinds of acid produced during the natural process of the souring of milk are not always the same. Lactic acid is generally predominant, but other acids of different germicidal value are often formed in larger or smaller quantity. At relatively high temperature, the bacilli of the *Bacillus coli* group are more active than at low temperature so that larger amounts of volatile acids are produced. Other products of bacteria may influence the results one way or the other. Finally, milk contains a large amount of organic compounds which absorb some of the acid formed and, consequently, cause the germicidal effect to be diminished. The so-called incubation period of milk is due in part to absorption by the casein of acid formed by bacteria.

Another difficulty not to be overlooked is the variable resistance of bacteria to germicidal agents. When a culture of typhoid bacilli comes in contact with a germicide which destroys the majority of cells but leaves an appreciable number of more highly resistant individuals, these multiply and leave the milk in a dangerous condition even though not present in sufficient numbers to permit detection with certainty. The variability of bacteria in their resistance to inimical conditions is well illustrated by the work of Ayers and Johnson,⁶ who found that some cells of *Streptococcus lacticus* and of *Bacillus coli* may survive pasteurization.

In consideration of these difficulties it seemed to me important to test the effect of sterilized mixtures of milk and lactic acid on pathogenic bacteria. It is true that by doing this some of the factors which

6. Jour. Agr. Research, 1914, 2, p. 321; 1915, 3, p. 401.

may be important are excluded. The influence of saprophytic bacteria and the influence of acid other than lactic acid are not brought into play. On the other hand, this method has the advantage of dealing with pure cultures so that all but a small number of the bacteria can be detected. It was found not practicable to count colonies in plates prepared from undiluted milk, as much precipitated casein was present which obscured colonies.

A series of experiments was carried out under the following conditions. Into each of eight 500 c.c. Erlenmeyer flasks were placed 300 c.c. certified milk from which about 90 percent of the cream had been removed. To these flasks pure lactic acid was added successively in the following amounts: 0.4 c.c. = 0.13 percent, 0.8 c.c. = 0.27 percent, 1.2 c.c. = 0.40 percent, 1.6 c.c. = 0.53 percent, 2.0 c.c. = 0.67 percent, 2.3 c.c. = 0.77 percent, 2.6 c.c. = 0.87 percent, and 2.9 c.c. = 0.97 percent. The flasks were then sterilized in an autoclave for fifteen minutes under fifteen pounds pressure.

These quantities of lactic acid were determined after a number of preliminary experiments. Since part of the lactic acid combines with the calcium of the casein, it is necessary to titrate the mixture of milk and acid after sterilization to determine the exact amount of active acid. It was apparent that in different lots of milk the amount of acid thus "lost" was not always the same. Titrations, therefore, had to be made with every fresh lot of milk. After titration six flasks were selected out of the eight so that as nearly as possible each flask contained a fairly regular amount of acid in excess of the previous flask. Titration was carried on with 0.05 N. NaOH with phenolphthalein as indicator. The smallest amount of acid was approximately that of raw milk. Some of the acid in raw milk is lost by heating so that the smallest amount of lactic acid added restored approximately the normal amount present in raw milk. Even this small amount caused precipitation of some of the casein during sterilization, as some of the acid must have combined with the calcium of the casein. The amount of acid in the succeeding flasks was increasingly larger, until the last flask contained about as much acid as is usually found in palatable buttermilk.

After the amount of acid was determined by titration, the flasks were inoculated with cultures of the bacteria to be tested. Plates were prepared after inoculation to determine the number of bacteria per c.c. The medium used for plating was beef extract agar of 1 percent acidity, except for *Bacillus diphtheriae*. In this case, 1 percent horse serum was added to the medium. The flasks were then incubated at 37 C. For three successive days the milk in each flask was titrated and plates prepared. It seemed sufficient to continue plating for three days, since buttermilk of greater age is rarely consumed. The plates were incubated for two days at 37 C. and then the colonies counted. The following bacteria were tested: *B. coli*, *B. dysenteriae* (Flexner), *B. typhosus*, *B. diphtheriae* (Park 8), *B. paratyphosus* B, *Spirillum cholera*. Cultures were prepared in broth and the same volume of the culture added to each flask. To each flask was added 1 c.c. of the culture of *B. coli*, 2 c.c. of the culture of *S. cholerae*, and 1.5 c.c. of each of the other four cultures. These volumes were decided upon after preliminary experiments so as to control to some extent the numbers inoculated.

The temperature for incubating the flasks was 37 C. This of course is higher than the temperature at which buttermilk is prepared. The multiplication of

pathogenic bacteria at 37 C. would naturally be greater than at the lower temperature at which buttermilk is made. This perhaps makes the experiments vary in a measure from natural conditions, but on the other hand germicides usually have greater effect at higher temperatures and possibly the difference is not great.

In Table 1, all the data are given.

Bacillus Coli.—In Flask 1, the initial acidity of 0.14 percent increased to 0.45 percent in two days and the number of bacteria increased from 1,610,000 to 410,000,000. On the third day the acidity was 0.53 percent. The number of bacteria had decreased slightly. Flasks 2 and 3, in the ratio of increase of acid and the number of bacteria, were similar to Flask 1. In Flask 4, the initial acidity was 0.52 percent, which after twenty-four hours had not increased. The number of bacilli after twenty-four hours had decreased materially, and after two and three days no bacilli could be found in a dilution of 1:10. In Flask 5, the result was similar to that of Flask 4, perhaps slightly more pronounced. In Flask 6, with 0.67 percent acidity, all bacteria were destroyed within twenty-four hours. The remarkable thing about this series is the fact that when the acidity was low at the time of inoculation, as in Flasks 1 and 2, the numbers were still high when 0.53-0.55 percent acid was present on the third day. But when the initial acidity was 0.52 percent or more, very few bacteria survived. The probable explanation is that among the cells inoculated there were some that were able to resist and multiply in greater acidity than other cells. These resistant cells became more tolerant to the acid as it developed, through their own metabolism, and we finally have as a result of multiplication a large number of highly resistant bacilli. If, however, inoculation took place in a flask with relatively high initial acidity, the cells of higher resistance had no chance to become accustomed to greater acidity and practically all died in relatively short time.

The limits of acid tolerance of *Bacillus coli* seem to be about 0.58-0.60 percent acid.

Bacillus Dysenteriae.—In Flask 1, there was considerable multiplication of bacilli within twenty-four hours at an acidity of 0.20 per cent. On the second day at 0.30 percent acidity, the number was practically the same; but on the third day there was a decided decrease. The limits of acid tolerance seem to be between 0.3 and 0.4 percent acid. In Flask 2, there was a rise in the number of bacteria and an increase of acid to 0.29 percent within two days. After this there was a decrease in bacterial numbers and a slight increase in acid. There is evidently a distinct relation between bacterial numbers and amount of acid when dealing with pure cultures. In the other four flasks, no bacteria were found after twenty-four hours. The initial acidity in these flasks was above the limits of tolerance. In Flasks 3 to 6, the initial number of bacteria was much smaller than in Flasks 1 and 2, altho the same volume of the same culture was added. It seems probable that the initial amount of acid was so great that a large number of bacilli were destroyed during the time elapsing between inoculation and plating, altho this was done with the utmost expedition. The initial number of bacteria became less as the amount of acid became greater in successive flasks.

Bacillus Typhosus.—In Flask 1, there was an increase after twenty-four hours and a decrease after two and three days. The increase of acidity from the second to the third day was slight, but reduction of the numbers of bacteria was considerable. Reduction of numbers commenced at 0.3 percent acid. In Flask 2, the highest amount of acid was reached on the third day. This

TABLE 1

RATE OF GROWTH OF BACTERIA IN STERILIZED MILK CONTAINING DIFFERENT AMOUNTS OF LACTIC ACID.

Organism	Num- ber of Flask	Percent- age of Lactic Acid Added	Titration				Number of Colonies			
			After Sterilization		One Day After Inoculation		Two Days After Inoculation		Three Days After Inoculation	
			nNaOH	Percentage of Lactic Acid	nNaOH	Percentage of Lactic Acid	nNaOH	Percentage of Lactic Acid	nNaOH	Percentage of Lactic Acid
B. coli...	1	0.13	1.5	0.14	3.5	0.31	5.0	0.45	5.9	0.53
	2	0.27	2.9	0.26	4.0	0.36	5.1	0.46	5.9	0.53
	3	0.40	3.9	0.35	5.8	0.52	5.8	0.52	6.1	0.55
	4	0.67	5.8	0.52	6.0	0.54	6.0	0.54	6.0	0.54
	5	0.77	6.7	0.60	6.8	0.61	6.7	0.60	6.8	0.61
	6	0.87	7.5	0.67	7.6	0.68	7.5	0.67	7.5	0.67
B. dysen- teriae	1	0.13	1.6	0.14	2.2	0.20	3.3	0.30	4.5	0.40
	2	0.27	3.0	0.27	2.9	0.26	3.2	0.29	3.9	0.35
	3	0.40	4.3	0.39	4.2	0.38	4.3	0.39	4.3	0.39
	4	0.67	5.2	0.47	5.3	0.48	5.3	0.48	5.4	0.49
	5	0.77	6.1	0.55	6.2	0.56	6.3	0.57	6.2	0.56
	6	0.87	7.6	0.68	7.5	0.68	7.4	0.67	7.4	0.67
B. typho- sus	1	0.13	1.5	0.14	3.3	0.30	4.3	0.39	4.5	0.41
	2	0.27	2.6	0.23	3.1	0.28	3.3	0.30	3.6	0.32
	3	0.40	3.6	0.32	3.9	0.35	3.4	0.31	3.4	0.31
	4	0.53	4.7	0.42	4.6	0.41	4.7	0.42	4.7	0.42
	5	0.77	5.8	0.52	5.9	0.53	6.0	0.54	6.0	0.54
	6	0.87	8.2	0.74	8.1	0.73	8.2	0.74	8.2	0.74
B. diph- theriae	1	0.13	1.5	0.14	1.8	0.16	1.8	0.16	1.8	0.16
	2	0.27	2.6	0.23	2.9	0.26	3.0	0.27	2.9	0.26
	3	0.53	3.6	0.32	3.6	0.32	3.6	0.32	3.4	0.31
	4	0.57	4.7	0.42	4.2	0.38	3.6	0.32	3.3	0.30
	5	0.77	5.9	0.53	5.8	0.52	6.0	0.54	5.9	0.53
	6	0.77	7.0	0.63	6.9	0.62	6.9	0.62	6.9	0.62
B. para- typhosus B	1	0.13	1.6	0.14	3.5	0.31	3.7	0.33	3.8	0.34
	2	0.27	3.1	0.28	3.4	0.31	4.1	0.37	4.2	0.38
	3	0.53	3.7	0.33	3.7	0.33	4.0	0.36	4.0	0.36
	4	0.67	5.9	0.53	5.8	0.52	5.7	0.51	5.8	0.52
	5	0.77	6.1	0.55	6.2	0.56	6.2	0.56	6.2	0.56
	6	0.87	7.9	0.71	7.8	0.71	7.9	0.71	7.9	0.71
Spirillum cholerae	1	0.13	1.2	0.11	1.4	0.13	1.3	0.12	1.3	0.12
	2	0.27	2.8	0.25	2.7	0.24	2.7	0.24	2.4	0.21
	3	0.53	3.7	0.33	3.8	0.34	3.7	0.33	3.7	0.33
	4	0.67	5.3	0.48	5.2	0.47	5.2	0.47	5.2	0.47
	5	0.77	5.9	0.53	5.9	0.53	5.8	0.52	5.9	0.53
	6	0.97	7.7	0.69	7.3	0.70	7.8	0.70	7.8	0.70

The lowest dilution plated was 1:10. Where "0" appears, it means that no colonies were found in this dilution.

amount, however, was but slightly higher than in Flask 1 after twenty-four hours. This may be explained by the survival and multiplication of acid tolerant forms, and it is worthy of notice that in Flask 2 the numbers increased to the end, reaching the large number of 609,000,000. Here again is evidence of a relation between bacterial numbers and the amount of acid. In Flask 3, we find a decrease after twenty-four hours with a slight increase in acidity. On the second and third days there was considerable increase. This can be explained by the destruction of the majority of cells under the influence of the initial amount of acid, leaving some cells of high acid tolerance. These multiplied in spite of the acid for some time. The amount of acid decreased somewhat on the second and third days, possibly due to the ability of this strain of typhoid bacilli to produce alkali in milk. In the fourth flask, nearly all bacilli were destroyed within twenty-four hours and none could be found after that. In Flasks 5 and 6, all bacilli were destroyed during the interval between inoculation and plating. The limits of acid tolerance of *Bacillus typhosus* lie between 0.3 and 0.4 percent acid.

Bacillus Diphtheriae.—In Flask 1, there was a steady increase in numbers of bacilli with a slight increase in acidity. In Flask 2, the increase in numbers was relatively small, as was also the increase in acidity. In Flask 3, we have at first a reduction in numbers and then a decided, tho relatively small, increase. The amount of acid remained unchanged. In Flask 4, no colonies were found after twenty-four hours in a dilution of 1:10. A few colonies, however, must have survived, since there were 2,330 after two days and over 9,000,000 after three days. The amount of acid was reduced from 0.42 to 0.30 percent. The strain of *Bacillus diphtheriae* (Park 8) which was used in these experiments is the same strain that is used widely for preparation of diphtheria toxin. During the incubation period of the toxin there is at first a formation of acid, followed by formation of alkali. Possibly this same process took place in the milk and the fact explains the decrease in acid. In Flask 5, one colony was found after twenty-four hours in a dilution of 1:10 and in Flask 6, none was found. There was considerable reduction of numbers of bacteria in Flasks 4, 5, and 6 during the interval between inoculation and plating. The limits of acid tolerance of *Bacillus diphtheriae* are between 0.27 and 0.32 percent acid.

Bacillus Paratyphosus B.—In Flask 1, there was an increase after twenty-four hours, followed by a decrease after two and three days. The acidity increased from 0.14 to 0.34 percent. In Flask 2, there was an increase after one day, but large numbers were destroyed immediately after inoculation. Some highly resistant cells must have survived, since after two days 3,000,000 were present. In Flasks 3 to 6, there were no colonies found after twenty-four hours. The reduction immediately after inoculation was decided in Flasks 2 to 6. This series was the second one, the results having been rather unexpected so that repetition seemed advisable. The limits of acid tolerance of *Bacillus paratyphosus B* are between 0.28 and 0.37 percent acid.

Spirillum Cholerae.—This organism is known to be sensitive to acids. In two successive series no colonies could be found either immediately after inoculation, or after one, two, and three days.

Under the conditions of the experiments reported, it would be hardly safe to assume that *Bacillus coli* is always destroyed in the presence of 0.6 percent lactic acid in milk. It is true that but few survived in the experiments, but the amount of acid which did not abso-

lutely destroy all bacilli in twenty-four hours was 0.6 percent. For *Bacillus dysenteriae* the amount of acid necessary to destroy all bacilli with certainty was above 0.4 percent, probably close to 0.42 percent. *Bacillus typhosus* seemed to be destroyed at about 0.42 percent, altho there was great reduction of numbers at 0.35 percent, or perhaps even below this figure. *Bacillus diphtheriae* was found alive in 0.32 percent acid, but not in 0.42 percent after twenty-four hours. *Bacillus paratyphosus* B seemed to be destroyed by 0.33 percent acid.

It seems safe to assume that all bacteria tested in these experiments are destroyed when the acidity of milk reaches 0.45 percent, except *Bacillus coli*. Since this organism is present in practically all market milk, its presence in buttermilk would not indicate danger. Buttermilk usually contains 0.6-0.9 percent acid. It might seem that with this amount of acid, buttermilk could be considered safe, even tho the original milk had been contaminated with pathogenic bacteria. The experiments, however, show that there is a possibility of strains of exceptional acid tolerance multiplying. Whether such strains are able to multiply in amounts of acid higher than those occurring in these experiments remains an open question. If the initial pollution is heavy, such a possibility exists and therefore too much reliance should not be placed on the safety of buttermilk unless prepared from pasteurized milk. The experiments indicate however that danger of infection from buttermilk is much smaller than from sweet raw milk.

The experiments were made with pure lactic acid at the outset. This condition was somewhat modified by acid produced by the metabolism of some of the bacteria, chiefly by *Bacillus coli*, and to some extent by the other test organisms except *Spirillum cholerae*. The amount of acid present, after incubation in those flasks of low initial acidity, was probably composed of lactic acid and some volatile acids so that the final acid was not strictly comparable to the acid in the flasks of higher initial acid. Whether pure lactic acid, or the resulting mixture of lactic acid and volatile acid, is of higher germicidal value is not clear from these experiments. This introduces a factor of uncertainty which must be reckoned with.

In buttermilk there are many bacteria besides lactic acid bacteria. The presence of these other bacteria may favor the multiplication of pathogenic bacteria by preparing, through their metabolism, food substances from the milk compounds which are more suitable for the nourishment of the pathogenic bacteria.

CONCLUSIONS

Some acid-tolerant cells of *Bacillus coli* may survive the presence of 0.6 percent lactic acid in milk.

B. dysenteriae, *B. typhosus*, *B. diphtheriae*, *B. paratyphosus* B, and *Spirillum cholerae* in these experiments were destroyed by the presence of 0.45 percent lactic acid. It is possible that strains of these bacteria exist which are able to resist a greater amount of lactic acid.

Acid-tolerant strains of *B. coli*, *B. dysenteriae*, *B. typhosus*, and *B. paratyphosus* B may multiply in the presence of quantities of lactic acid which are destructive to the majority of cells. The smaller the initial amount of lactic acid, the more likely is the growth of acid-tolerant strains. Consequently, the slower milk sours, the greater is the danger of pathogenic bacteria surviving.

The growth of the test bacteria is influenced to a marked degree by the amount of acid present. Up to a fairly definite amount of acid there is an increase in numbers, followed by a decrease, which becomes more pronounced as the amount of acid increases. The amount of acid may increase after the number of bacteria has commenced to decrease owing to the liberation of enzymes.

Acids other than lactic acid are frequently present in buttermilk. Buttermilk, therefore, should be looked upon with suspicion, especially if heavily polluted, unless prepared from pasteurized milk. Still the chances of buttermilk becoming a carrier of infection are much smaller than of raw sweet milk.

The presence of saprophytic bacteria in buttermilk may have some influence on pathogenic bacteria. Whether this influence is favorable or otherwise, is difficult to determine by present bacteriological methods.

THE VACUUM METHOD OF DRAWING ANTIHOG- CHOLERA SERUM *

THOS. P. HASLAM, A. E. HAGAN, AND R. V. CHRISTIAN

(From the Veterinary Department of the Kansas State Experiment Station, Manhattan, Kans.)

Through a series of experiments a system has been devised whereby blood may be rapidly and aseptically drawn from the tail of a hog by means of a vacuum. This technic is of great importance in the manufacture of the Dorset-Niles antihog-cholera serum, as there are more than one hundred institutions in the United States as well as numerous plants in other parts of the world manufacturing this antiserum. In this paper a brief description of a specially constructed instrument for vacuum tail bleeding, a satisfactory method for the restraint of the hog during bleeding, and an efficient method of separating the defibrinated blood from the fibrin will be given.

RESTRAINT

Any method of restraint in which the hog is held in an upright position is satisfactory unless several are to be bled at the same time by each workman. In this case, it is important that the animal be securely held. By the crate illustrated in Figure 1, the hog is so well restrained that very little attention is necessary during the process of bleeding. Special features that deserve attention are: (a) The hog is lifted completely off its feet. (b) Its back is pressed firmly against the top of the crate. (c) Its nose is held securely.

BLEEDING

After the usual preparation, the hyperimmune is placed in the crate and the entire tail as well as a small area round the root of the tail is washed, shaved, and thoroughly disinfected. One attendant then firmly grasps the tail with a pair of sterile forceps, while the second clips off the end of the tail and removes a plug of sterile cotton from the mouth of the bleeding apparatus. The tail is guided into the open mouth of the bleeding top by means of the forceps and the top pushed up until the mouth touches the body at the root of the tail.

* Received for publication April 10, 1915.

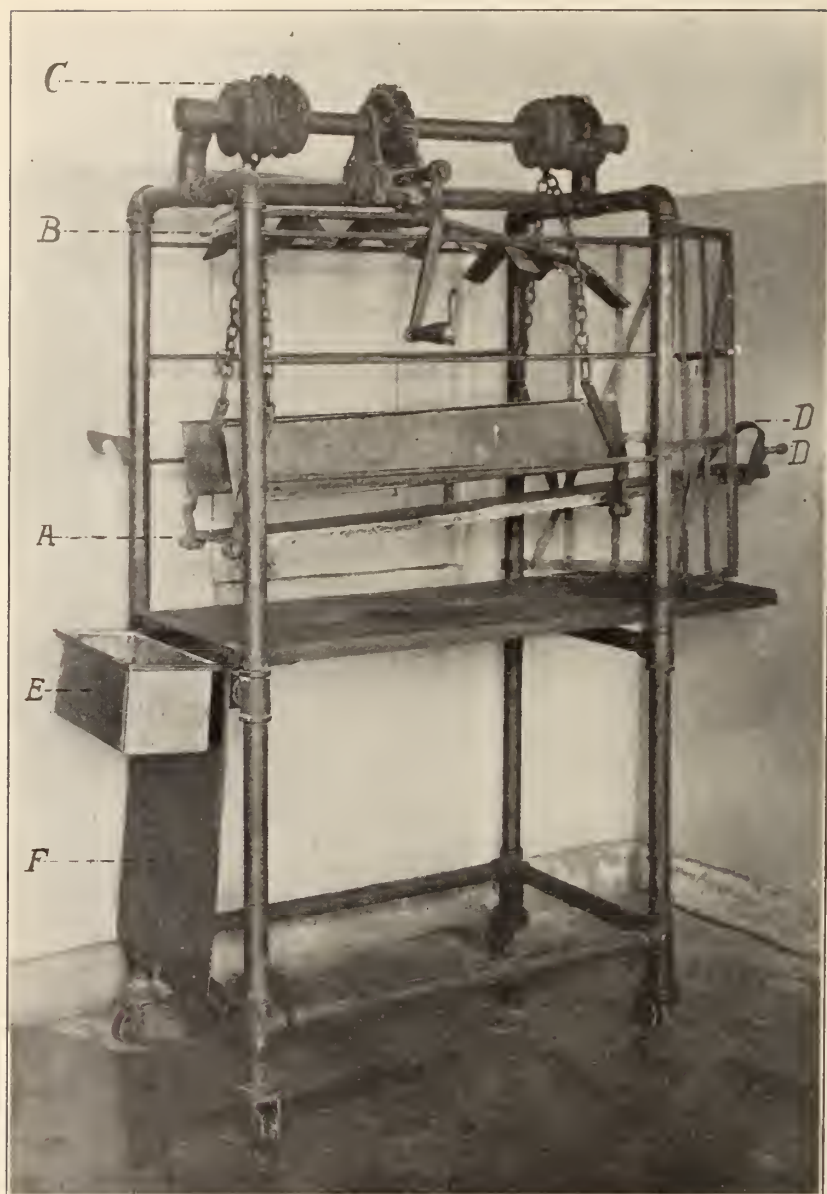


Fig. 1.—Bleeding and hypering crate. A. Carriage for hog, partially raised. B. Roof against which hog is drawn. C. Windlass for raising carriage. D. Nose holder adjusted by crank—D.¹ E. Removable pan for droppings. F. Back of crate used in loading.



Fig. 2.—Bleeding top on jar. A. Base. B. Mouthpiece. C. Rubber connection. D. Lug for retaining wire. E. Male adapter for connecting with cotton air filter. F. Ordinary fruit jar rubber.

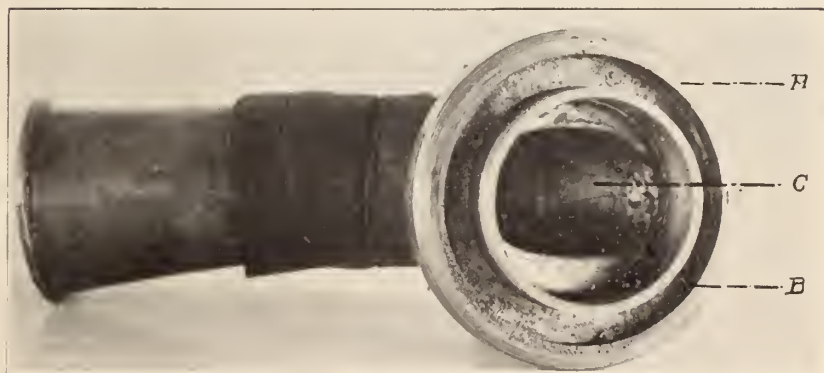


Fig. 3.—Under side of base. A. Bearing surface which rests in top of jar. B. Protecting rim which enters top of jar. C. Opening through which end of the tail enters the jar.

This is accomplished without touching the tail with the hands, or in any way contaminating any portion of it. The jar is then exhausted by means of a vacuum pump. As the vacuum increases, the flow of blood materially increases until a maximum is reached; above this degree of vacuum an increase produces a decrease in the rate of bleeding. The maximum vacuum to be used varies considerably for different individuals. A vacuum sufficient to bleed one hog may be secured from an ordinary water pump. A small rotary vacuum pump

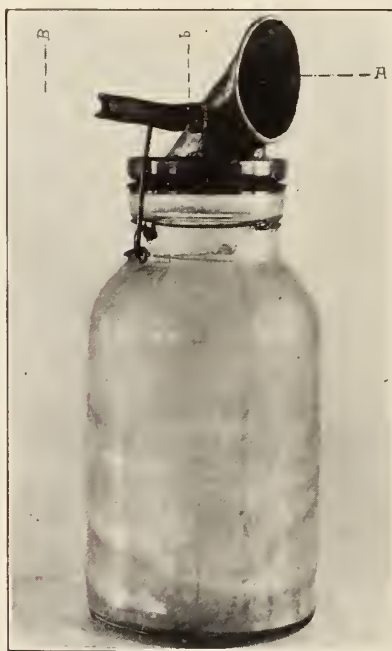


Fig. 4.—Bleeding top for short tailed hog. A. Mouthpiece direct connected with base. B. Air filter connected by female adapter to male adapter.

driven by an electric motor furnishes a satisfactory vacuum. This pump will maintain a vacuum of about twenty-eight inches of mercury. After the bleeding is started and the vacuum regulated, the blood will usually flow freely until the required quantity is drawn. In rare instances it has been found necessary to withdraw the tail and clip off another small piece, the same technic being employed as in the original operation. There is a noteworthy difference in the speed of

bleeding dependent upon the length of the tail, and consequently the size of the vessels severed. When the large blood vessels within a few inches of the body are severed, bleeding is extremely profuse.

A number of bacteriological determinations upon the blood of normal hogs, bled by the vacuum method, showed the blood drawn by this method to be without noteworthy contamination.

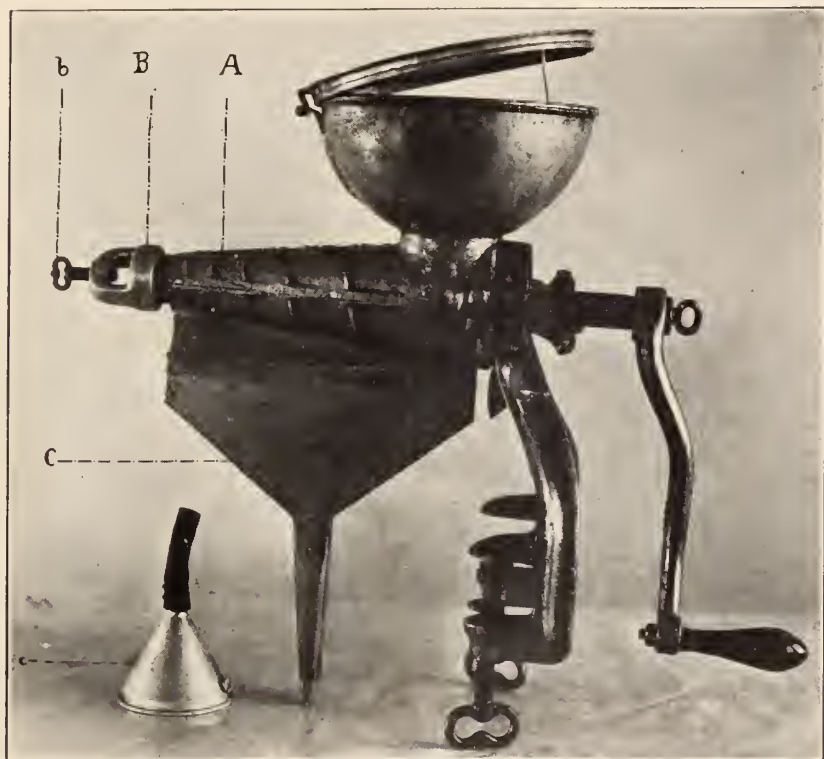


Fig. 5.—Modified juicer used for defibrinating. The lid and funnel have been added to the original machine and essential changes have been made in the interior of machine. A. Barrel in which great pressure is obtained by a spiral screw. B. Discharge for fibrin regulated by the screw (b). C. Funnel through which the defibrinated blood flows. The small protective apron (c) is connected to the bottom of the funnel C by the rubber tube attached to (c).

Different views of the bleeding apparatus are shown in Figures 2 and 3. Figure 4 shows the bleeding top modified for hogs with short tails. This cut also shows the sterile air filter B to which the vacuum line is connected. This air filter was omitted from Figure 2 in order to show the male adapter E to which the filter is connected by means of a female adapter.

DEFIBRINATING AND STRAINING

For defibrinating, a fruit juicer (Fig. 5) with certain modifications is used. The blood drawn from the tail by vacuum is first gently shaken until coagulation is complete. Then the whole content of the jar is emptied into the "juicer." By slowly turning the handle of the juicer the defibrination is completed and the fibrin and the defibrinated blood are separated. Straining through gauze removes some small bits of fibrin which pass through the juicer.

The technic just described is the result of considerable experimentation and has now been in daily use in the production of antihog-cholera serum for more than six months. The trial thus far shows that the yield of serum is increased, the labor of bleeding and defibrination is reduced, and the quality of the serum greatly improved. During this time some minor defects have presented themselves, but no serious defects have become apparent.

FURTHER OBSERVATIONS ON THE BACTERIOLOGY OF RHINITIS WITH SPECIAL REFERENCE TO AN ANAEROBIC ORGANISM (BACILLUS RHINITIS) *

RUTH TUNNICLIFF

(From the Memorial Institute for Infectious Diseases, Chicago.)

In a previous article¹ I described a delicate, curved, gram negative, anaerobic organism, which was observed in the early stages of acute rhinitis, before the discharge became purulent. Altho this organism resembled a spirochete in some respects, it is probably a bacillus. I would suggest naming it *Bacillus rhinitis*.

This bacillus was observed in smears in thirty cases of acute coryza, in three cases of chronic coryza, and in one case of chronic pharyngitis. It was seen only once in twenty normal noses. The organism was isolated in pure culture eight times. It produced a rhinitis in the human subject and in a dog. Changes occurred in the opsonic index to this organism, the index being low during the acute stage and rising as the infection subsided.

On account of the frequency with which this bacillus was observed in this series of cases, it was thought it might be of interest to determine its prevalence in a larger number of cases and during different seasons of the year. Smears from the nose were made and stained with carbol-gentian-violet. The bacilli were present in five of sixty-three smears made from the normal nose. Forty-seven percent of these smears contained no bacteria. Smears from the nose in fifty cases of acute coryza while the discharge was mucoid in character, showed the *bacillus rhinitis* in all but one. In 66 percent of these smears this organism was the only one observed. It was not seen in sixteen cases of purulent rhinitis, in scarlet fever, and diphtheria. It was present in seventeen of the eighteen cases of chronic rhinitis in which the discharge was not purulent. It was not seen in seven chronic cases with purulent secretion. The combination of these results with my previous observations shows that the bacilli were present in 6 percent of normal noses, 98 percent of the cases of acute rhinitis, and 90 percent of the chronic cases with mucoid discharges.

* Received for publication April 14, 1915.

1. Jour. of Infect. Dis., 1913, 13, p. 283.

All of the smears from the nose were carefully examined for fusiform bacilli on account of the resemblance at times between the two organisms in cultures. The bacillus fusiformis was not observed in any of the acute cases, and only three times in cases of chronic purulent rhinitis. The two organisms are readily distinguished in smears, as seen in the specimen from the pharynx, in pharyngitis, where the two may be seen side by side. The bacillus rhinitis does not stain so distinctly as the fusiform bacillus; it is also more curved and is not fusiform. They can be distinguished also in mixed cultures from the throat, where the bacillus fusiformis is always present. The bacillus rhinitis is flexible and has a slightly progressive motion which is different from the vibratory motion of the bacillus fusiformis.

The opsonic index in patients immunized with Bacillus rhinitis was found to be high to seven strains of this organism, but normal to two strains of the bacillus fusiformis isolated from cases of Vincent's angina. The same results were obtained with the serum of a patient recovering from an acute rhinitis. Complement-fixation experiments made by Howell,² showed with the serum of one acute case of rhinitis a strong fixation with the bacillus rhinitis, but no fixation with the bacillus fusiformis. Another case examined at the end of a long attack of rhinitis, in which smears and cultures were not made, showed strongly positive reaction to both. The sera of three patients immunized with the bacillus rhinitis showed positive fixation with the three strains of the bacillus rhinitis and no fixation with the bacillus fusiformis. A patient with Vincent's angina gave a positive fixation with the bacillus fusiformis and no fixation with the bacillus rhinitis.

On account of the difficulty of always distinguishing between the bacillus rhinitis and cilia, anaerobic cultures were made in twenty-six cases. The external nares being first washed with 95 percent alcohol, the secretion was collected on sterile gauze, on a swab, or in a Petri dish. The discharge was collected from the nasopharynx in some of the chronic cases and washed three times in sterile salt solution. Cultures were made on goat blood agar, the agar being slightly alkaline in reaction, and in ascites broth (1:3), the broth being made from Fairchild's culture peptone. Sterile tissue was at times added to the media, but did not appear to add to its efficacy. Ascites fluid was used with the goat blood in some of the blood agar slants. It was often difficult to grow and to isolate this bacillus in pure culture. It was

2. Jour. Infect. Dis., 1915, 16, p. 456.

grown in seventeen of the twenty-one acute cases and isolated in pure culture nine times. The organism was isolated in pure culture from the sputum in two of these cases with accompanying bronchitis. Pure cultures were obtained from all of the five chronic cases, in which the organisms were present in large numbers. The organism has not been grown in cultures from the normal nose.

Aerobic cultures, on goat blood agar, with and without ascites fluid, were also made to obtain some idea as to the number and kinds of other bacteria present. A colony or two of the staphylococcus albus appeared in almost every case. There were a few colonies of diphtheroid bacilli in five cases. The streptococcus pyogenes was isolated once. The streptococcus viridans grew twice and the bacillus mucosus three times in the anaerobic broth cultures. In a few cases in which aerobic bacterial antigens gave positive fixation, the corresponding bacteria were not found in the smears or culture. On the other hand, according to Howell's observations, complement fixation was generally obtained with the sera of persons with acute rhinitis when the bacillus rhinitis was employed as the antigen.

The results of human immunization experiments with vaccines of the bacillus rhinitis will be reported later.

CONCLUSION

The bacillus rhinitis appears then to have some etiologic relation to acute and chronic rhinitis on account of its almost constant presence in the nose in such cases, its general absence from the normal nose, its ability to produce rhinitis experimentally with recovery in pure culture, and on account of the production, in cases of acute and chronic rhinitis and in persons injected with the bacillus, of specific antibodies (opsonins and complement-binding bodies).

GENERAL INDEX

GENERAL INDEX

PAGE

Abderhalden Test, The Production, Through Immunization, of Specific Ferments Against Bacteria, as Detected by - - - - -	313
Acid Formed in Milk and Cream, Relation of the Number of Streptococcus Lacticus to the Amount of - - - - -	285
Agar, Starch, a Useful Culture Medium - - - - -	385
Agglutination and the Fermentation Reactions Among the Streptococci, A Study of the Correlation of - - - - -	327
AIRD, J. A., and MEYER, K. F. Various Sporotricha Differentiated by the Fermentation of Carbohydrates. Studies on American Sporotrichosis, I	399
Amboceptor, Natural Antisheep, The Technic of the Wassermann Reaction with Reference to Thomas and Ivy's Method of Complement Dosage and to the Management of - - - - -	119
Antidiphtheric Horse Serum, On the Specific Precipitin in the Blood of Persons Injected with - - - - -	63
Antitoxin, Tetanus, Individual and Group Variation in Guinea-Pigs in the American Method of Testing - - - - -	410
Appendicitis, The Bacteriology of, and its Production by Intravenous Injection of Streptococci and Colon Bacilli (<i>with Plates 12-16</i>) - - -	240
ARKIN, AARON. The Influence of an Oxidizing Substance (Sodium Iodoxybenzoate) on Immune Reactions - - - - -	349
Bacilli, Colon, The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and - - - - -	240
Bacilli, Tubercle, Sodium Tellurite as a Rapid Test for the Viability of. Studies in the Biochemistry and Chemotherapy of Tuberculosis, XII -	47
Bacilli, Tubercle, of the Human and the Bovine Type, Simultaneous Infection in a Child with - - - - -	361
Bacilli, Typhoid, Sensitized and Non-Sensitized, On the Relative Virulence of - - - - -	26
Bacillus Bulgaricus, A Study of the So-Called Implantation of - - - -	210
Bacillus Diphtheriae, Bacteriemia Due to - - - - -	292
Bacillus Rhinitis, Further Observations on the Bacteriology of Rhinitis, with Special Reference to an Anaerobic Organism - - - - -	493
Bacillus Tetanus, Experimental Study of the Distribution and Habitat of	132
Bacillus Welchii, Effect of Symbiosis upon Spore Formation by, with Special Reference to the Presence of These Spores in Stools - - -	35
Bacillus Welchii Group of Bacteria, Classification of - - - - -	31
Bacteria, Bacillus Welchii Group of, Classification of - - - - -	31
Bacteria, The Production, Through Immunization, of Specific Ferments Against, as Detected by the Abderhalden Test - - - - -	313
Bacteriemia Due to Bacillus Diphtheriae - - - - -	292
Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli - - - - -	240
BESCHE, ARENT DE. Simultaneous Infection in a Child with Tubercle Bacilli of the Human and the Bovine Type - - - - -	361
Biochemistry and Chemotherapy of Tuberculosis, Studies on, XII. The Action of Sodium Sulphocyanate in Tuberculosis, 38; XIII. Sodium Tellurite as a Rapid Test for the Viability of Tubercle Bacilli - - -	47

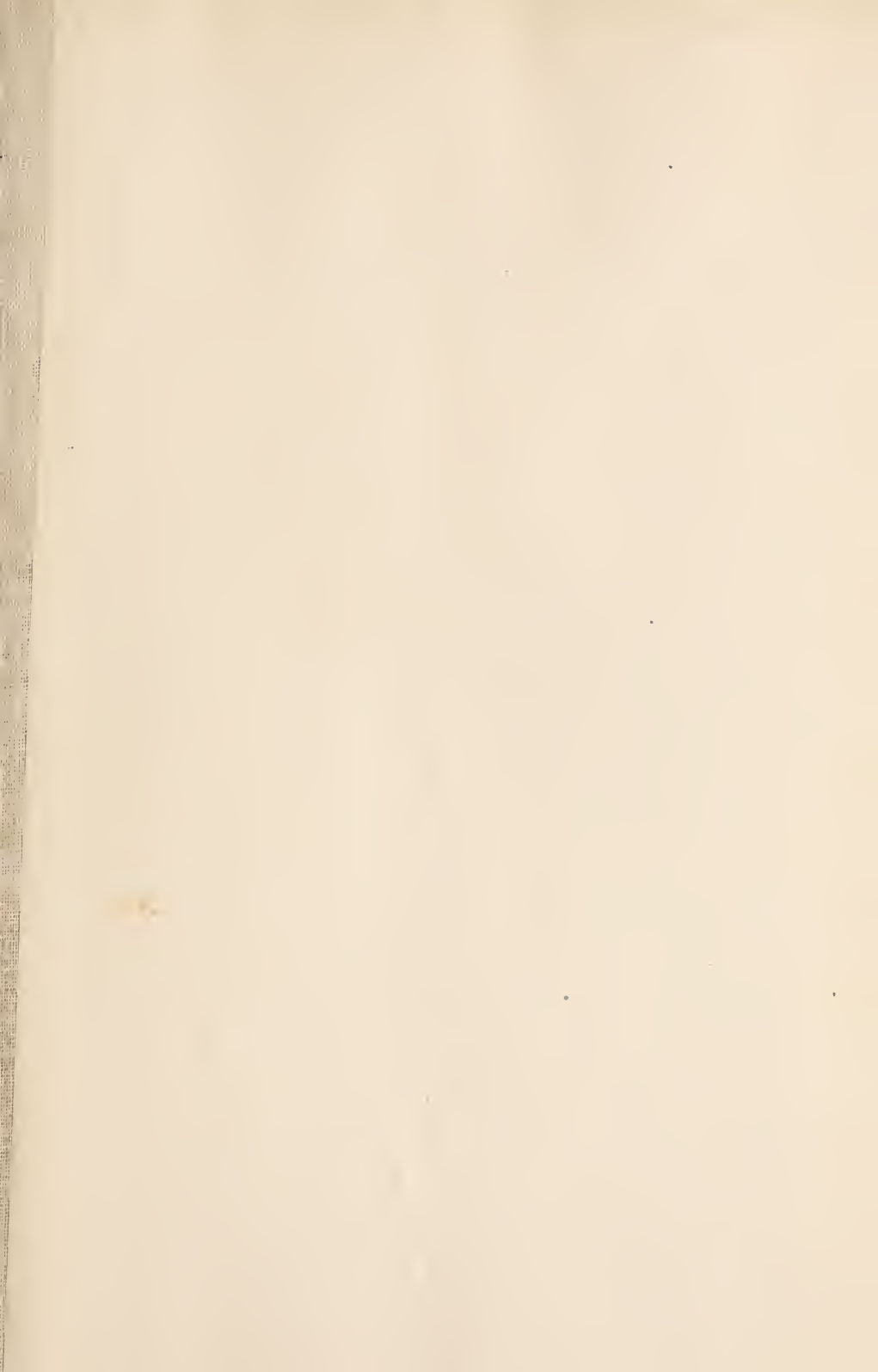
Blood of Persons Injected with Antidiphtheric Horse Serum, On the Specific Precipitin in - - - - -	63
Blood Parasites, A Method of Transmitting - - - - -	311
Blood Serum in Infectious Diseases, The Ferment Activity of - - -	466
Bulgaricus, Bacillus, A Study of the So-Called Implantation of - - -	210
Carcinoma, in Mice, A Comparison of the Immunizing Effects of the Subcutaneous and Intraperitoneal Administrations of Tumor Cells Against the Growth of - - - - -	122
Carbohydrates, Various Sporotricha Differentiated by Fermentation of - -	399
CASSELMAN, ARTHUR J., and KOLMER, JOHN A. Natural Hemolysins in Human Serum - - - - -	441
CECIL, RUSSELL L. On the Relative Virulence of Sensitized and Non-Sensitized Typhoid Bacilli - - - - -	26
Chemotherapy and Biochemistry of Tuberculosis, Studies on, XII. The Action of Sodium Sulphocyanate in Tuberculosis, 38; XIII. Sodium Tellurite as a Rapid Test for the Viability of Tubercle Bacilli - - -	47
China, On the Spirochetal Infection of Ulcers in - - - - -	269
Cholera, hog-, Studies on. Inoculation Experiment with Pure Culture of Spirochaeta Hyos - - - - -	54
Cholera Serum, Antihog, The Vacuum Method of Drawing - - - - -	487
CHRISTIAN, R. V., HASLAM, THOS. P., and HAGAN, A. E. The Vacuum Method of Drawing Antihog Cholera Serum - - - - -	487
Classification of the Bacillus Welchii Group of Bacteria - - - - -	31
Colon Bacilli, The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and - - - - -	240
Complement Fixation in Acute Rhinitis - - - - -	456
Complement Dosage, The Technic of the Wassermann Reaction with Reference to Thomas and Ivy's Method of, and the Management of Natural Antisheep Amboceptor - - - - -	119
Complement Fixation in the Diagnosis of Gonococcal Infections - - -	303
Complement Fixation in Whooping Cough - - - - -	389
Conglutination in the Diagnosis of Dourine (Trypanosomiasis of the Horse)	461
CORPER, HARRY J. The Action of Sodium Sulphocyanate in Tuberculosis. Studies on the Biochemistry and Chemotherapy of Tuberculosis, XII, 38; Sodium Tellurite as a Rapid Test for the Viability of Tubercle Bacilli. Studies on the Biochemistry and Chemotherapy of Tuberculosis, XIII - - - - -	47
Cream, Relation of the Number of Streptococcus Lacticus to the Amount of Acid Formed in Milk and - - - - -	285
DEWEY, KAETHE, and LE COUNT, E. R. Syphilitic Leptomeningitis (<i>with Plates 1-11</i>) - - - - -	142
Diagnosis of Gonococcal Infections, Complement Fixation in the - - -	303
Diagnostic Value, The, of Intracutaneous Injection of Diphtheria Toxin (Schick Reaction) - - - - -	342
Diets, The Fecal Flora of Typhoid Fever and Its Reaction to Various - -	72
Diphtheriae Bacillus, Bacteriemia Due to - - - - -	292
Diphtheria Toxin (Schick's Reaction), The Diagnostic Value of Intracutaneous Injection of - - - - -	342
Dourine (Trypanosomiasis of the Horse) Conglutination in the Diagnosis of - - - - -	461
DRAKE, RAYMOND H., and KING, WALTER E. Inoculation Experiment with Pure Culture of Spirochaeta Hyos - - - - -	54

	PAGE
EGGERS, H. E. On the Spirochetal Infection of Ulcers in China - - -	269
Erythema Nodosum, The Etiology and Experimental Production of (<i>with Plates 17-22</i>) - - - - -	367
FALLS, FREDERICK HOWARD. The Ferment Activity of the Blood Serum in Infectious Diseases - - - - -	466
Fecal Flora of Typhoid Fever and Its Reaction to Various Diets - - -	72
Ferment Activity of the Blood Serum in Infectious Diseases - - -	466
Fermentation of Carbohydrates, Various Sporotricha Differentiated by -	399
Fermentation Reactions Among the Streptococci, A Study of the Correlation of the Agglutination and the - - - - -	327
Ferments, Specific, for the Typhoid-Coli Group, The Production and Detection of - - - - -	319
Ferments, Specific, Against Bacteria as Detected by the Abderhalden Test, The Production, Through Immunization of - - - - -	313
FLEISHER, MOYER S., and SEELIG, M. G. A Comparison of the Immunizing Effects of the Subcutaneous and Intraperitoneal Administrations of Tumor Cells Against the Growth of Carcinoma in Mice - - -	122
Flora, Fecal, of Typhoid Fever and Its Reaction to Various Diets - - -	72
Glycerin Resistance of Various Organisms, Studies on the Cultivation of the Virus of Vaccinia III, with a Note on - - - - -	205
Gonococcal Infections, Complement Fixation in the Diagnosis of - - -	303
Gonococcus, Studies on, III - - - - -	426
GRUND, MARIE, and STEINHARDT, EDNA. Studies on the Cultivation of the Virus of Vaccinia III, with a Note on the Glycerin Resistance of Various Organisms - - - - -	205
HAGAN, A. E., HASLAM, THOS. P., and CHRISTIAN, R. V. The Vacuum Method of Drawing Antihog Cholera Serum - - - - -	487
HANSEN, PAUL, and PARKER, HORATIO N. Typhoid Fever in Rockford, Illinois - - - - -	1
HASLAM, THOS. P., HAGAN, A. E., and CHRISTIAN, R. V. The Vacuum Method of Drawing Antihog Cholera Serum - - - - -	487
HEINEMANN, P. G. Relation of the Number of Streptococcus Lacticus to the Amount of Acid Formed in Milk and Cream, 285; The Germicidal Effect of Lactic Acid in Milk, 479; The Variability of Two Strains of Streptococcus Lacticus - - - - -	221
Hemolysins, Natural, in Human Serum - - - - -	441
Hog-Cholera, Studies on. Inoculation Experiment with Spirochaeta Hyos, 54; The Vacuum Method of Drawing Antihog-Cholera Serum - - -	487
HOWELL, KATHARINE. Complement Fixation in Acute Rhinitis - - -	456
Hyos, Spirochaeta, Inoculation Experiment with Pure Culture of - - -	54
Illinois, Typhoid Fever in Rockford - - - - -	1
Immune Reactions, The Influence of an Oxidizing Substance (Sodium Idioxybenzoate) on - - - - -	349
Immunization, The Production of Specific Ferments Against Bacteria Through - - - - -	313
Immunizing Effects of the Subcutaneous and Intraperitoneal Administration of Tumor Cells Against the Growth of Carcinoma in Mice, A Comparison of - - - - -	122
Implantation, So-Called, of the Bacillus Bulgaricus, A Study of - - -	210
IRONS, ERNEST E., and NICOLL, H. N. Complement Fixation in the Diagnosis of Gonococcal Infections - - - - -	303
KING, WALTER E., and DRAKE, RAYMOND H. Inoculation Experiment with Pure Culture of Spirochaeta Hyos. Studies on Hog-Cholera - - -	54

	PAGE
KITE, G. L., and WHERRY, W. B. The Mechanism of Phagocytosis - - -	109
KLIGLER, I. J. A Study of the Correlation of the Agglutination and Fermentation Reactions Among the Streptococci - - -	327
KOLMER, JOHN A. A Method of Transmitting Blood Parasites, 311; Natural Hemolysins in Human Serum (WITH ARTHUR J. CASSELMAN)	441
KRUMWEIDE, CHARLES, JR., and MANN, ALICE G. The Effect of Quinin on Rabies - - -	24
Lactic Acid in Milk, The Germicidal Effect of - - -	479
LE COUNT, E. R., and DEWEY, KATHE. Syphilitic Leptomenigitis - -	142
Leptomenigitis, syphilitic - - -	142
MANN, ALICE G., and KRUMWEIDE, CHARLES, JR. The effect of Quinin on Rabies - - -	24
MAHER, LORETTA, and WEAVER, GEORGE H. The Diagnostic Value of Intracutaneous Injection of Diphtheria Toxin (Schick's Reaction) - -	342
Mechanism, The, of Phagocytosis - - -	109
Method, A, of Transmitting Blood Parasites - - -	311
MEYER, K. F., and AIRD, J. A. Various Sporotricha Differentiated by the Fermentation of Carbohydrates. Studies on American Sporotrichosis, I - - -	399
Milk and Cream, Relation of the Number of Streptococcus Lacticus to the Amount of Acid Formed in - - -	285
MOON, V. H. Further Observations on the Effect of Quinin in Rabies -	58
NICOLL, H. K., and IRONS, ERNEST E. Complement Fixation in the Diagnosis of Gonococcal Infections - - -	303
NOBLE, WILLIS. Experimental Study of the Distribution and Habitat of the Tetanus Bacillus - - -	132
OTTENBERG, REUBEN, and FRAZIER, BLANCHE. The Technic of the Wassermann Reaction with Reference to Thomas and Ivy's Method of Complement Dosage and to the Management of Natural Antisheep Amboceptor - - -	119
Paragaertner Organism, An Epidemic Simulating Typhoid Caused by -	448
Parasites, Blood, A Method of Transmitting - - -	311
PARKER, HORATIO N., and HANSEN, PAUL. Typhoid Fever at Rockford, Illinois - - -	1
Phagocytosis, The Mechanism of - - -	109
Precipitin, Specific, in the Blood of Persons Injected with Antidiphtheric Horse Serum - - -	63
Quinin, The Effect of, on Rabies, 24; Further Observations on - -	58
Rabies, The Effect of Quinin on, 24; Further Observations on - -	58
RAHE, ALFRED H. A Study of the So-Called Implantation of the Bacillus Bulgaricus - - -	210
Rhinitis, Acute, Complement Fixation in - - -	456
Rhinitis, Further Observations on the Bacteriology of, with Special Reference to an Anaerobic Organism (Bacillus Rhinitis) - - -	493
ROBINSON, GEORGE H. An Epidemic, Simulating Typhoid, Caused by a Paragaertner Organism - - -	448
Rockford, Illinois, Typhoid Fever in - - -	1
ROSENOW, EDWARD C. The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli (with Plates 12-16), 240; The Etiology and Experimental Production of Erythema Nodosum (with Plates 17-22) - - -	367

	PAGE
Schick Reaction - - - - -	343
SEELIG, M. G., and FLEISHER, MOYER S. A Comparison of the Immunizing Effects of the Subcutaneous and Intraperitoneal Administrations of Tumor Cells Against the Growth of Carcinoma in Mice - - -	122
Serum, Horse, Antidiphtheric, On the Specific Precipitin in the Blood of Persons Injected with - - - - -	63
Serum, Antihog Cholera, The Vacuum Method of Drawing - - -	487
Serum, Blood, in Infectious Diseases, The Ferment Activity of - - -	466
Serum, Human, Natural Hemolysins in - - - - -	441
SIMONDS, J. P. Classification of the Bacillus Welchii Group of Bacteria, 31; The Effect of Symbiosis upon Spore Formation by Bacillus Welchii, with Special Reference to the Presence of These Spores in Stools -	35
SMITH, GEORGE H. The Production, Through Immunization, of Specific Ferments Against Bacteria as Detected by the Abderhalden Test, 313; The Production and Detection of Specific Ferments by the Typhoid-Coli Group - - - - -	319
Sodium Iodoxybenzoate, an Oxidizing Substance, The Influence of, on Immune Reactions - - - - -	349
Sodium Sulphocyanate in Tuberculosis, The Action of. Studies on the Biochemistry and Chemotherapy of Tuberculosis, XII - - -	38
Sodium Tellurite as a Rapid Test for the Viability of Tubercle Bacilli. Studies on the Biochemistry and Chemotherapy of Tuberculosis, XIII	47
Spirochaeta Hyos, Inoculation Experiment with Pure Culture of. Studies on Hog-Cholera - - - - -	54
Spirochetal Infection of Ulcers in China - - - - -	269
Spore Formation, Effect of Symbiosis upon, by Bacillus Welchii, with Special Reference to the Presence of These Spores in Stools - -	35
Sporotricha, Various, Differentiated by the Fermentation of Carbohydrates. Studies on American Sporotrichosis, I - - - - -	399
Sporotrichosis, American, Studies on, I - - - - -	399
Starch Agar, a Useful Culture Medium - - - - -	385
STEINHARDT, EDNA, and GRUND, MARIE. Studies on the Cultivation of the Virus of Vaccinia III, with a Note on the Glycerin Resistance of Various Organisms - - - - -	205
Streptococci, A Study of the Agglutination and the Fermentation Reactions Among the - - - - -	327
Streptococci, The Bacteriology of Appendicitis and Its Production by Intravenous Injection of, and Colon Bacilli (<i>with Plates 12-16</i>) - - -	240
Streptococcus Lacticus, Relation of the Number of, to the Amount of Acid Formed in Milk and Cream - - - - -	285
Streptococcus Lacticus, The Variability of Two Strains of - - -	221
Symbiosis, Effect of, upon Spore Formation by Bacillus Welchii, with Special Reference to the Presence of These Spores in Stools - -	35
Syphilitic Leptomeningitis (<i>with Plates 1-11</i>) - - - - -	142
TABER, LOREN B. Individual and Group Variation in Guinea-Pigs in the American Method of Testing Tetanus Antitoxin - - - - -	410
Tetanus Antitoxin, Individual and Group Variation in Guinea-Pigs in the American Method of Testing - - - - -	410
Tetanus Bacillus, Experimental Study of the Distribution and Habitat of	132
Thomas and Ivy's Method of Complement Dosage. The Technic of the Wassermann Reaction with Reference to, and to the Management of Natural Antisheep Amboceptor - - - - -	119
Toxin, Diphtheria, The Diagnostic Value of Intracutaneous Injection of -	342

	PAGE
TORREY, JOHN C. The Fecal Flora of Typhoid Fever and Its Reactions to Various Diets - - - - -	72
Trypanosomiasis of the Horse, Dourine, Conglutination in the Diagnosis of Tubercle Bacilli of the Human and the Bovine Type, Simultaneous Infection in a Child with - - - - -	461
Tubercle Bacilli, Sodium Tellurite as a Rapid Test for the Viability of - - - - -	361
Tuberculosis, Studies on the Biochemistry and Chemotherapy of, XII. The Action of Sodium Sulphocyanate in, 38; XIII. Sodium Tellurite as a Rapid Test for the Viability of Tubercle Bacilli - - - - -	47
Tumor Cells, A Comparison of the Immunizing Effects of the Subcutaneous and Intraperitoneal Administrations of, Against the Growth of Carcinoma in Mice - - - - -	122
TUNNICLIFF, RUTH. Further Observations on the Bacteriology of Rhinitis, with Special Reference to an Anaerobic Organism (Bacillus Rhinitis) - - - - -	493
Typhoid, An Epidemic Simulating, Caused by a Paragaertner Organism - - - - -	448
Typhoid Bacilli, Sensitized and Non-Sensitized, On the Relative Virulence of - - - - -	26
Typhoid-Coli Group, The Detection and Production of Specific Ferments for - - - - -	319
✓ Typhoid Fever in Rockford, Illinois - - - - -	1
Typhoid Fever, The Fecal Flora of, and Its Reaction to Various Diets - - - - -	72
Ulcers in China, On the Spirochetel Infection of - - - - -	269
Vaccinia III, Studies on the Cultivation of the Virus of, with a Note on the Glycerin Resistance of Various Organisms - - - - -	205
Vacuum Method of Drawing Antihog Cholera Serum - - - - -	487
Variability, The, of Two Strains of Streptococcus Lacticus - - - - -	221
Variation, Individual and Group, in Guinea-Pigs in the American Method of Testing Tetanus Antitoxin - - - - -	410
VEDDER, EDWARD B. Starch Agar, a Useful Culture Medium - - - - -	385
Virulence, Relative, of Sensitized and Non-Sensitized Typhoid Bacilli - - - - -	26
Virus of Vaccinia III, Studies on the Cultivation of, with a Note on the Glycerin Resistance of Various Organisms - - - - -	205
WADE H. WINDSOR. Bacteriemia Due to Bacillus Diphtheriae - - - - -	292
WARDEN, CARL C. Studies on the Gonococcus, III - - - - -	426
Wassermann Reaction, The Technic of, with Reference to Thomas and Ivy's Method of Complement Dosage and to the Management of Natural Antisheep Amboceptor - - - - -	119
WEAVER, GEORGE H., and MAHER, LORETTA. The Diagnostic Value of Intracutaneous Injection of Diphtheria Toxin (Schick Reaction) - - - - -	342
WEHRBEIN, HEINRICH. Conglutination in the Diagnosis of Dourine (Trypanosomiasis of the Horse) - - - - -	461
WELLS, CLIFFORD W. On the Specific Precipitin in the Blood of Persons Injected with Antidiphtheric Horse Serum - - - - -	63
WHERRY, W. B., and KITE, G. L. The mechanism of Phagocytosis - - - - -	109
Whooping Cough, Complement Fixation in - - - - -	389
WINHOLT, WALTER. Complement Fixation in Whooping Cough - - - - -	389



COLUMBIA UNIVERSITY LIBRAR

